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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003904813 for a patent by FUJISAWA PHARMACEUTICAL CO., LTD and WAKUNAGA PHARMACEUTICAL CO., LTD as filed on 04 September 2003.

WITNESS my hand this
Seventeenth day of October 2003

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

Fujisawa Pharmaceutical Co., Ltd.
and
Wakunaga Pharmaceutical Co., Ltd.

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Cephem Compounds"

The invention is described in the following statement:

DESCRIPTION

CEPHEM COMPOUNDS

TECHNICAL FIELD

The present invention relates to new cephem compounds and pharmaceutically acceptable salts thereof. More particularly, the present invention relates to new cephem compounds and pharmaceutically acceptable salts thereof, which have antimicrobial activities, to processes for preparation thereof, to pharmaceutical composition comprising the same, and to a method for treating infectious diseases in human being and animals.

DISCLOSURE OF INVENTION

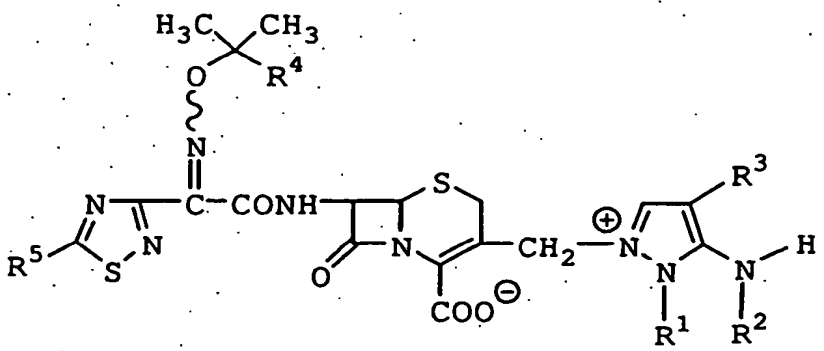
One object of the present invention is to provide novel cephem compounds and pharmaceutically acceptable salts thereof, which are highly active against a number of pathogenic microorganisms.

Another object of the present invention is to provide processes for the preparation of said cephem compounds and salts thereof.

A further object of the present invention is to provide a pharmaceutical composition comprising, as an active ingredient, said cephem compounds or their pharmaceutically acceptable salts.

Still further object of the present invention is to provide a method for treating infectious diseases caused by pathogenic microorganisms, which comprises administering said cephem compounds to infected human being or animals.

The object cephem compounds of the present invention are novel and can be represented by the following general formula [I]:



[I]

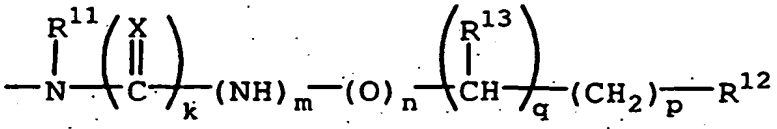
wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen or amino protecting group, or

R^1 and R^2 are bonded together and form lower alkylene;

R^3 is



wherein X is O or NH,

R^{11} is hydrogen or amino protecting group,

R^{12} is amino or protected amino,

R^{13} is hydrogen or hydroxy,

k, m, n and q are independently 0 or 1, and

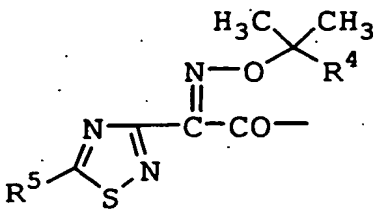
p is 0, 1 or 2;

R^4 is carboxy or protected carboxy; and

R^5 is amino or protected amino.

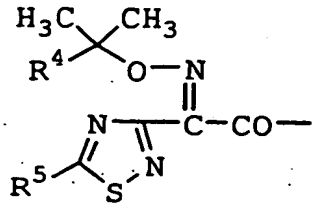
As to the object compound [I], the following points are to be noted.

That is, the object compound [I] includes syn isomer (Z form), anti isomer (E form) and a mixture thereof. Syn isomer (Z form) means one geometrical isomer having the partial structure represented by the following formula:



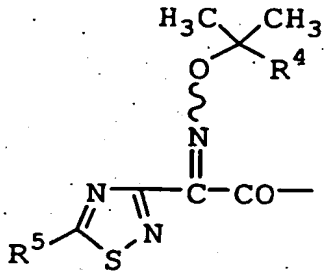
wherein R^4 and R^5 are each as defined above,

and anti isomer (E form) means the other geometrical isomer having the partial structure represented by the following formula:



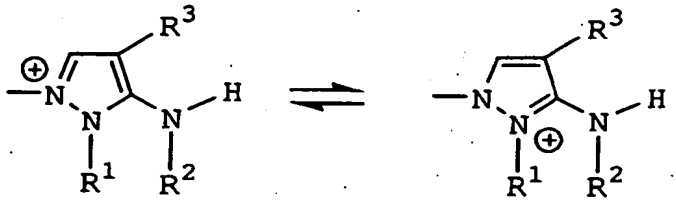
wherein R^4 and R^5 are each as defined above, and all of such geometrical isomers and mixture thereof are included within the scope of this invention.

In the present specification and claims, the partial structure of these geometrical isomers and mixture thereof are represented for convenience' sake by the following formula:



wherein R^4 and R^5 are each as defined above.

Another point to be noted is that the pyrazolio moiety of the compound [I] can also exist in the tautomeric form, and such tautomeric equilibrium can be represented by the following formula.



(A) (B)

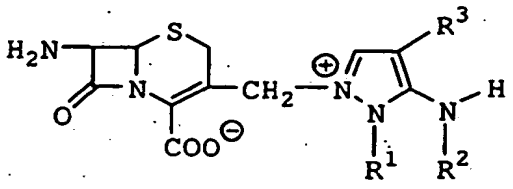
wherein R^1, R^2 and R^3 are each as defined above.

Both of the above tautomeric isomers are included within the scope of the present invention, and in the present specification and claims, however, the object compound [I] is represented for convenience' sake by one

expression of the pyrazolio group of the formula (A).

The cephem compound [I] of the present invention can be prepared by the following processes as illustrated in the following.

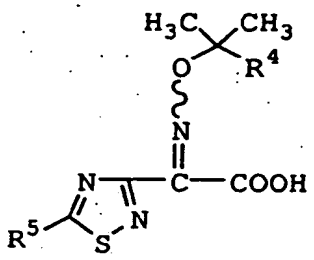
Process 1



[II]

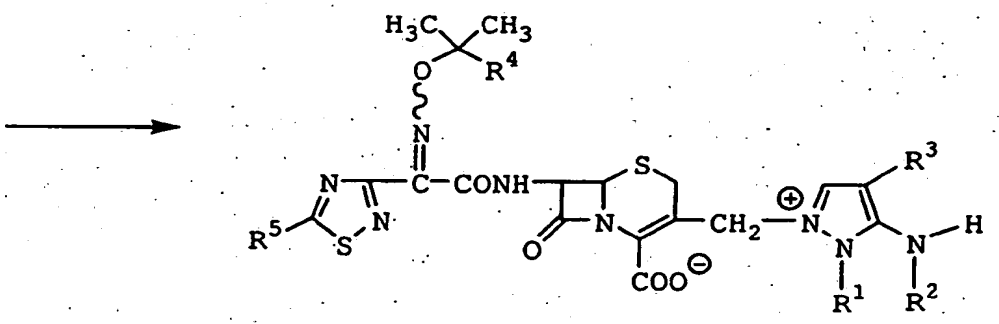
or its reactive derivative at the amino group, or a salt thereof

+



[III]

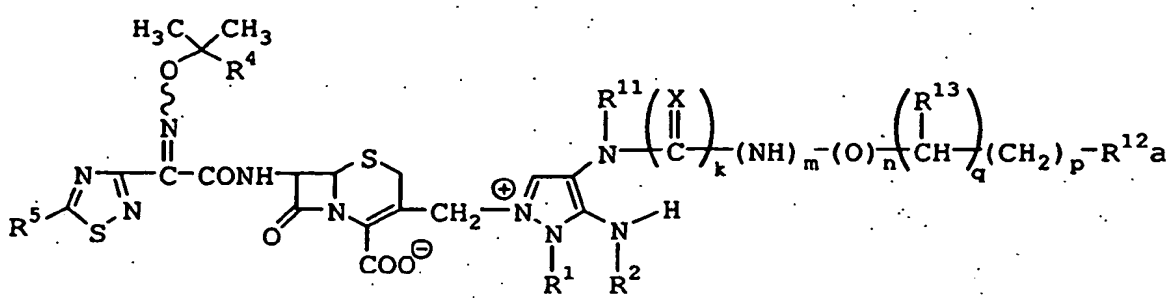
or its reactive derivative at the carboxy group, or a salt thereof



[I]

or a salt thereof

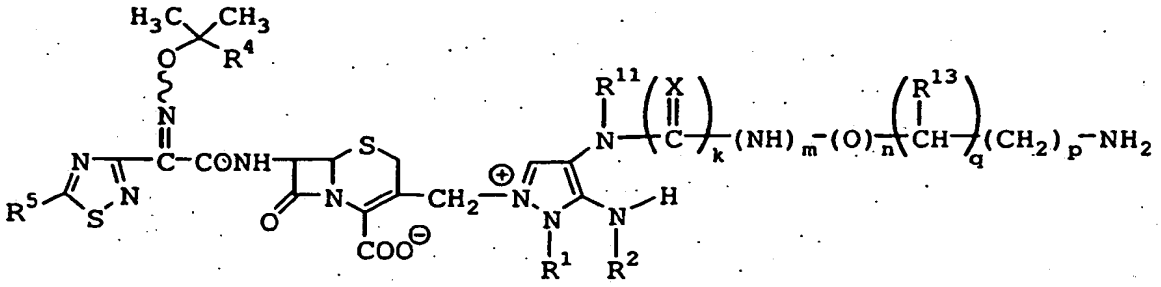
Process 2



[Ia]

or a salt thereof

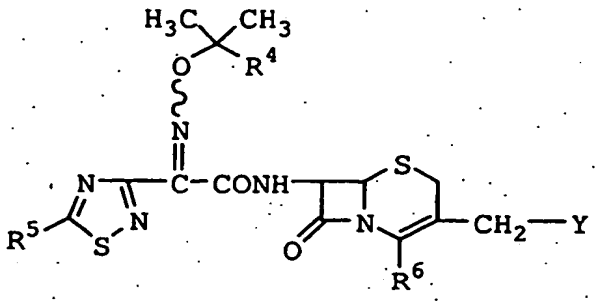
Elimination reaction of the amino protecting group



[Ib]

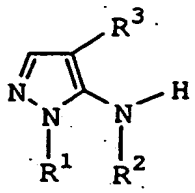
or a salt thereof

Process 3



[VI]

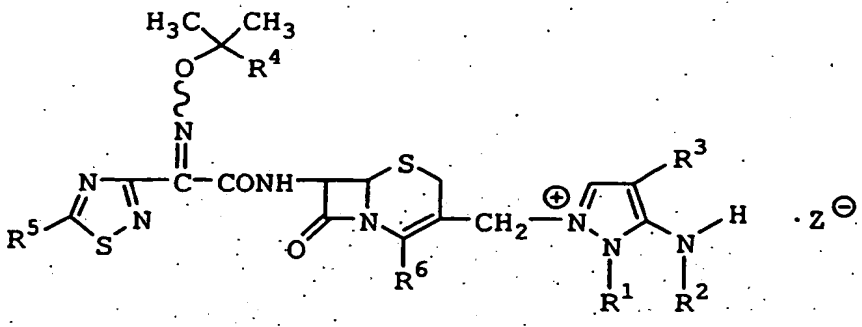
or a salt thereof



[VII]

or a salt thereof

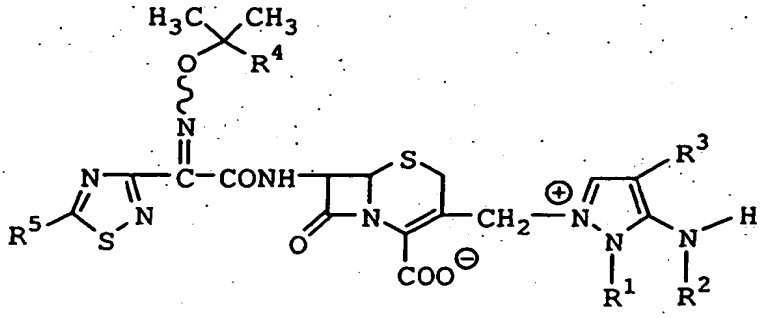
(i)



[VIII]

or a salt thereof

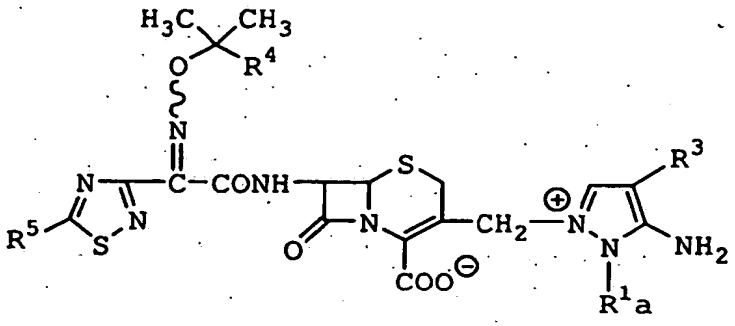
(ii)



[I]

or a salt thereof

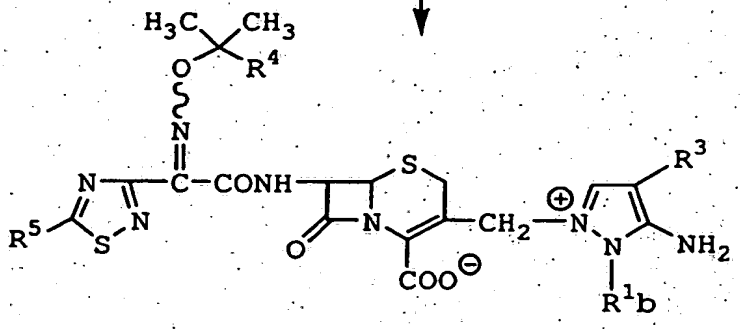
Process 4



[Ic]

or a salt thereof

Elimination rection of the hydroxy protecting group



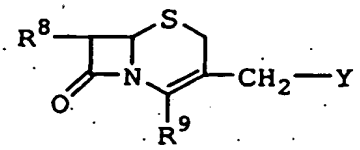
[Id]

or a salt thereof

wherein R^1, R^2, R^3, R^4, R^5, R^{11}, R^{13}, X, k, m, n, p and q are each as defined above,
R^6 is protected carboxy,
Y is a leaving group,
$Z^{\ominus}$ is an anion,
R^1a is protected hydroxy(lower)alkyl,
R^1b is hydroxy(lower)alkyl, and
$R^{12}a$ is protected amino.

The starting compounds [II] and [VI] can be prepared by the following processes.

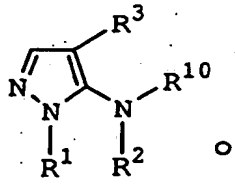
Process A



[X]

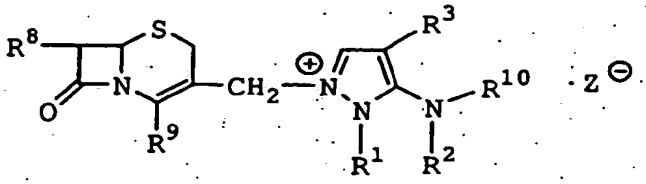
or a salt thereof

(i)



[XI]

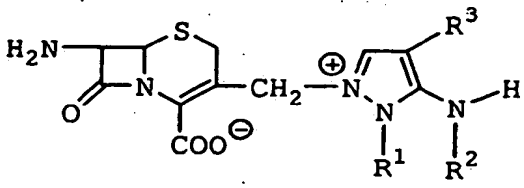
or a salt thereof



[XII]

or a salt thereof

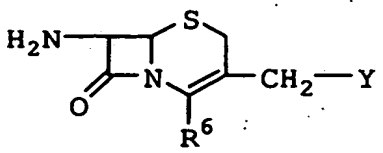
(ii)



[II]

or a salt thereof

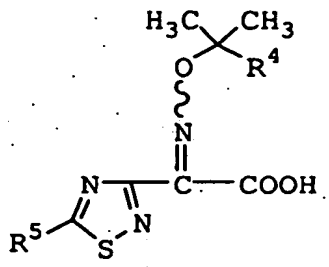
Process B



[XIII]

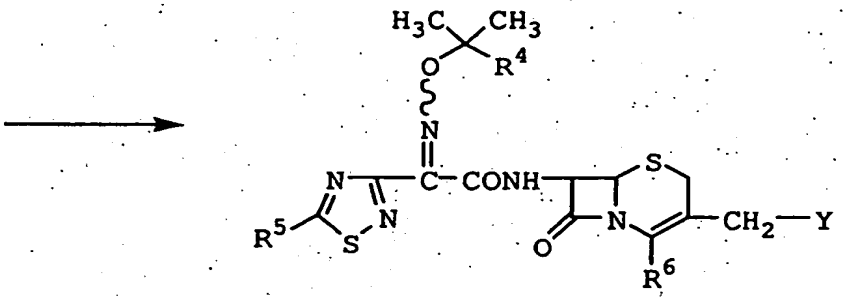
or its reactive derivative at the amino group, or a salt thereof

+



[XIV]

or its reactive derivative at the carboxy group, or a salt thereof



[VI]

or a salt thereof

wherein R^1, R^2, R^3, R^4, R^5, R^6, Y and $Z^{\ominus}$ are each as defined above,

R^8 is protected amino,

R^9 is protected carboxy, and

R^{10} is amino protecting group.

The starting compounds [VII] and [XI] or salts thereof can be prepared by the methods disclosed in the Preparations 3-46 and 48-53 described later or similar manners thereto.

In the above and subsequent descriptions of this specification, suitable examples of the various definitions are explained in detail as follows.

The term "lower" is used to mean a group having 1

to 6, preferably 1 to 4, carbon atoms, unless otherwise indicated.

Suitable "lower alkyl" and "lower alkyl" moiety in "hydroxy(lower)alkyl", "protected hydroxy(lower)alkyl" and "aryl(lower)alkyl", include straight or branched alkyl having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, tert-pentyl and hexyl, in which more preferred one is C_1-C_4 alkyl.

Suitable "hydroxy(lower)alkyl" includes hydroxy(C_1-C_6)alkyl such as hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 4-hydroxybutyl, 5-hydroxypentyl and 6-hydroxyhexyl, in which more preferred one is hydroxy(C_1-C_4)alkyl.

Suitable "lower alkylene" formed by R^1 and R^2 includes straight alkylene having 1 to 6, preferably 2 to 4 carbon atoms, such as methylene, ethylene, trimethylene and tetramethylene, in which more preferred one is straight alkylene having 2 or 3 carbon atoms.

Suitable "aryl" moiety in "aryl(lower)alkyl" includes C_6-C_{12} aryl such as phenyl and naphthyl, in which more preferred one is phenyl.

Suitable "aryl(lower)alkyl" includes mono-, di- or triphenyl(lower)alkyl such as benzyl, phenethyl, benzhydryl and trityl.

Suitable "lower alkanoyl" and "lower alkanoyl" moiety in "lower alkanoylamino" include straight or branched alkanoyl having 1 to 6 carbon atoms, such as formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl and hexanoyl, in which more preferred one is C_1-C_4 alkanoyl.

Suitable "lower alkoxy" moiety in "lower alkoxycarbonyl" and "lower alkoxycarbonylamino" includes straight or branched alkoxy having 1 to 6 carbon atoms, such as methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, tert-butoxy, pentyloxy, tert-pentyloxy and hexyloxy, in which more preferred one is

C_1-C_4 alkoxy.

Suitable "amino protecting group" in "protected amino" includes an acyl group as mentioned below, substituted or unsubstituted aryl(lower)alkylidene [e.g., benzylidene, hydroxybenzylidene, etc.], aryl(lower)alkyl such as mono-, di- or triphenyl(lower)alkyl [e.g., benzyl, phenethyl, benzhydryl, trityl, etc.], and the like.

Suitable "acyl" includes lower alkanoyl [e.g., formyl, acetyl, propionyl, hexanoyl, pivaloyl, etc.], mono(or di or tri)halo(lower)alkanoyl [e.g., chloroacetyl, trifluoroacetyl, etc.], lower alkoxycarbonyl [e.g., methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl, tert-pentyloxycarbonyl, hexyloxycarbonyl, etc.], carbamoyl, aroyl [e.g., benzoyl, toluoyl, naphthoyl, etc.], aryl(lower)alkanoyl [e.g., phenylacetyl, phenylpropionyl, etc.], aryloxycarbonyl [e.g., phenoxycarbonyl, naphthyloxycarbonyl, etc.], aryloxy(lower)alkanoyl [e.g., phenoxyacetyl, phenoxypropionyl, etc.], arylglyoxyloyl [e.g., phenylglyoxyloyl, naphthylglyoxyloyl, etc.], aryl(lower)alkoxycarbonyl which optionally substituted by suitable substituent(s) [e.g., benzyloxycarbonyl, phenethyloxycarbonyl, p-nitrobenzyloxycarbonyl, etc.], and the like.

Preferable examples of "amino protecting group" include aryl(lower)alkyl and acyl, in which more preferred ones are aryl(lower)alkyl, lower alkanoyl and lower alkoxycarbonyl, and particularly preferred ones are mono-, di- or triphenyl(C_1-C_6)alkyl, C_1-C_6 alkanoyl and (C_1-C_6)alkoxycarbonyl.

Preferable examples of "protected amino" include aryl(lower)alkylamino and acylamino, in which more preferred ones are aryl(lower)alkylamino, lower alkanoylamino and lower alkoxycarbonylamino, and particularly preferred ones are mono-, di- or triphenyl(C_1-C_6)alkylamino, C_1-C_6 alkanoylamino and (C_1-C_6)alkoxycarbonylamino.

Suitable "protected hydroxy" in the "protected hydroxy(lower)alkyl" includes acyloxy group, aryl(lower)alkyloxy group, and the like. Suitable "acyl" moiety in the "acyloxy" includes lower alkanoyl [e.g., formyl, acetyl, propionyl, hexanoyl, pivaloyl, etc.], mono(or di or tri)halo(lower)alkanoyl, [e.g., chloroacetyl, trifluoroacetyl, etc.], lower alkoxycarbonyl, [e.g., methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl, tert-pentyloxycarbonyl, hexyloxycarbonyl, etc.], carbamoyl, and the like. Suitable "aryl(lower)alkyl" moiety in the "aryl(lower)alkyloxy" includes mono-, di- or triphenyl(lower)alkyl [e.g., benzyl, phenethyl, benzhydryl, trityl, etc.], and the like.

Suitable "protected carboxy" includes esterified carboxy and the like, and concrete examples of esterified carboxy include lower alkoxycarbonyl [e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, pentyloxycarbonyl, hexyloxycarbonyl, 1-cyclopropylethoxycarbonyl, etc.] which may have suitable substituent(s), for example, lower alkanoyloxy(lower)alkoxycarbonyl [e.g., acetoxymethoxycarbonyl, propionyloxymethoxycarbonyl, butyryloxymethoxycarbonyl, valeryloxymethoxycarbonyl, pivaloyloxymethoxycarbonyl, 1-acetoxyethoxycarbonyl, 1-propionyloxyethoxycarbonyl, 2-propionyloxyethoxycarbonyl, hexanoyloxymethoxycarbonyl, etc.], lower alkanesulfonyl(lower)alkoxycarbonyl, [e.g., 2-mesylethoxycarbonyl, etc.] or mono(or di or tri)halo(lower)alkoxycarbonyl [e.g., 2-iodoethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, etc.]; lower alkenyloxycarbonyl [e.g., vinyloxycarbonyl, allyloxycarbonyl, etc.]; lower alkynyloxycarbonyl [e.g., ethynyloxycarbonyl, propynyloxycarbonyl, etc.]; aryl(lower)alkoxycarbonyl which may have suitable substituent(s) [e.g., benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 4-nitrobezyloxycarbonyl,

phenethyloxycarbonyl, trityloxycarbonyl, benzhydryloxycarbonyl, bis(methoxyphenyl)methoxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 4-hydroxy-3,5-di-tert-butylbenzyloxycarbonyl, etc.]; aryloxycarbonyl which may have suitable substituent(s) [e.g., phenoxycarbonyl, 4-chlorophenoxycarbonyl, tolyloxycarbonyl, 4-tert-butylphenoxycarbonyl, xylyloxycarbonyl, mesityloxycarbonyl, cumenyloxycarbonyl, etc.]; and the like.

Preferable examples of "protected carboxy" include lower alkoxycarbonyl and aryl(lower)alkoxycarbonyl which may have suitable substituent(s), in which more preferred one is (C_1-C_6)alkoxycarbonyl.

Suitable "leaving group" includes halogen [e.g., chlorine, bromine, iodine, etc.] or acyloxy such as arylsulfonyloxy [e.g., benzenesulfonyloxy, tosyloxy, etc.], lower alkylsulfonyloxy [e.g., mesyloxy, etc.], lower alkanoyloxy [e.g., acetyloxy, propionyloxy, etc.], and the like.

Suitable "anion" includes formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, chloride, bromide, iodide, sulfate, phosphate, and the like.

Suitable pharmaceutically acceptable salts of the object compound [I] are conventional non-toxic salts and include, for example, a salt with a base or an acid addition salt such as a salt with an inorganic base, for example, an alkali metal salt [e.g., sodium salt, potassium salt, etc.], an alkaline earth metal salt [e.g., calcium salt, magnesium salt, etc.], an ammonium salt; a salt with an organic base, for example, an organic amine salt [e.g., trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, etc.]; an inorganic acid addition salt [e.g., hydrochloride, hydrobromide, sulfate, phosphate, etc.]; an organic carboxylic or sulfonic acid addition salt

[e.g., formate, acetate, trifluoroacetate, maleate, tartrate, citrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.]; and a salt with a basic or acidic amino acid [e.g., arginine, aspartic acid, glutamic acid, etc.].

The preferred embodiments of the cephem compound of the present invention represented by the general formula [I] are as follows.

(1) The compound of the formula [I] wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen, aryl(lower)alkyl or acyl, or

R^1 and R^2 are bonded together and form lower alkylene;

R^4 is carboxy or esterified carboxy;

R^5 is amino or acylamino;

R^{11} is hydrogen or acyl; and

R^{12} is amino or acylamino,

or a pharmaceutically acceptable salt thereof.

(2) The compound of (1) above wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen, aryl(lower)alkyl, lower alkanoyl or lower alkoxycarbonyl, or

R^1 and R^2 are bonded together and form lower alkylene;

R^4 is carboxy or lower alkoxycarbonyl;

R^5 is amino, lower alkanoylamino or lower alkoxycarbonylamino;

R^{11} is hydrogen, lower alkanoyl or lower alkoxycarbonyl; and

R^{12} is amino, lower alkanoylamino or lower alkoxycarbonylamino,

or a pharmaceutically acceptable salt thereof.

(3) The compound of (2) above wherein

R^1 is C_1-C_6 alkyl or hydroxy(C_1-C_6)alkyl, and

R^2 is hydrogen, mono-, di- or triphenyl(C_1-C_6)alkyl, C_1-C_6 alkanoyl or (C_1-C_6)alkoxycarbonyl, or

R^1 and R^2 are bonded together and form C_1-C_6 alkylene;

R^4 is carboxy or (C_1-C_6)alkoxycarbonyl;

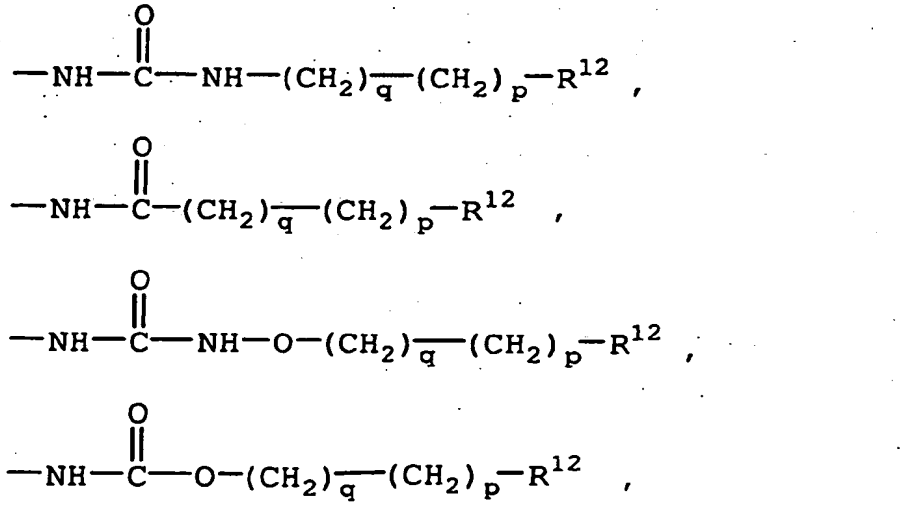
R^5 is amino, C_1-C_6 alkanoylamino or (C_1-C_6)alkoxycarbonylamino;

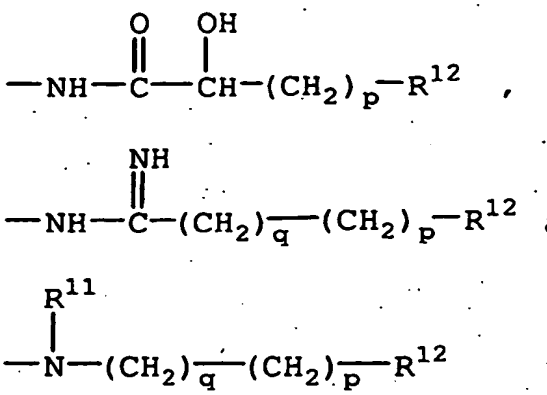
R^{11} is hydrogen, C_1-C_6 alkanoyl or (C_1-C_6)alkoxycarbonyl;
and
R^{12} is amino, C_1-C_6 alkanoylamino or
(C_1-C_6)alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

(4) The compound of (2) above wherein
R^1 is lower alkyl or hydroxy(lower)alkyl, and
R^2 is hydrogen, or
R^1 and R^2 are bonded together and form lower alkylene;
R^4 is carboxy;
R^5 is amino;
R^{11} is hydrogen or lower alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

(5) The compound of (4) above wherein
R^1 is C_1-C_6 alkyl or hydroxy(C_1-C_6)alkyl, and
R^2 is hydrogen, or
R^1 and R^2 are bonded together and form C_1-C_6 alkylene;
R^4 is carboxy;
R^5 is amino;
R^{11} is hydrogen or C_1-C_6 alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

(6) The compound of the formula [I] wherein





wherein R^{11}, R^{12}, p and q are each as defined above,
or a pharmaceutically acceptable salt thereof.

(7) The compound of (6) above wherein
R^{11} is hydrogen, lower alkanoyl or lower alkoxycarbonyl; and
R^{12} is amino, lower alkanoylamino or lower alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

(8) The compound of (7) above wherein
R^{11} is hydrogen, (C_1-C_6)alkanoyl or (C_1-C_6)alkoxycarbonyl; and
R^{12} is amino, (C_1-C_6)alkanoylamino or (C_1-C_6)alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

(9) The compound of (7) above wherein
R^{11} is hydrogen or lower alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

(10) The compound of (9) above wherein
R^{11} is hydrogen or (C_1-C_6)alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

The processes for preparing the object compound of the present invention are explained in detail in the following.

Process 1

The compound [I] or a salt thereof can be prepared by reacting the compound [II] or its reactive derivative

at the amino group, or a salt thereof with the compound [III] or its reactive derivative at the carboxy group, or a salt thereof.

Suitable reactive derivative at the amino group of the compound [II] includes Schiff's base type imino or its tautomeric enamine type isomer formed by the reaction of the compound [II] with a carbonyl compound such as aldehyde, ketone and the like; a silyl derivative formed by the reaction of the compound [II] with a silyl compound such as bis(trimethylsilyl)acetamide, mono(trimethylsilyl)acetamide [e.g., N-(trimethylsilyl)acetamide], bis(trimethylsilyl)urea and the like; a derivative formed by the reaction of the compound [II] with phosphorus trichloride or phosgene.

Suitable salts of the compound [II] and its reactive derivative can be referred to the ones as exemplified for the compound [I].

Suitable reactive derivative at the carboxy group of the compound [III] includes an acid halide, an acid anhydride, an activated amide, and an activated ester. A suitable example of the reactive derivatives may be an acid chloride; an acid azide; a mixed acid anhydride with an acid such as substituted phosphoric acid [e.g., dialkylphosphoric acid, phenylphosphoric acid, diphenylphosphoric acid, dibenzylphosphoric acid, halogenated phosphoric acid, etc.], dialkylphosphorous acid, sulfurous acid, thiosulfuric acid, sulfuric acid, alkanesulfonic acid [e.g., methanesulfonic acid, etc.], aliphatic carboxylic acid [e.g., acetic acid, propionic acid, butyric acid, isobutyric acid, pivalic acid, pentanoic acid, isopentanoic acid, 2-ethylbutyric acid, trichloroacetic acid, etc.] and aromatic carboxylic acid [e.g., benzoic acid, etc.]; a symmetrical acid anhydride; an activated amide with imidazole, 4-substituted imidazole, dimethylpyrazole, triazole or tetrazole; an activated ester [e.g., cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl [$(CH_3)_2N^+=CH-$]

ester, vinyl ester, propargyl ester, p-nitrophenyl ester, 2,4-dinitrophenyl ester, trichlorophenyl ester, pentachlorophenyl ester, mesylphenyl ester, phenylazophenyl ester, phenyl thioester, p-nitrophenyl thioester, p-cresyl thioester, carboxymethyl thioester, pyranyl ester, pyridyl ester, piperidyl ester, 8-quinolyl thioester, etc.]; or an ester with an N-hydroxy compound [e.g., N,N-dimethylhydroxylamine, 1-hydroxy-2-(1H)-pyridone, N-hydroxysuccinimide, N-hydroxyphthalimide, N-hydroxy-1H-benzotriazole, etc.]. These reactive derivatives can optionally be selected from them according to the kind of the compound [III] to be used.

Suitable salts of the compound [III] and its reactive derivative can be referred to the ones as exemplified for the compound [I].

The reaction is usually carried out in a conventional solvent such as water, alcohol [e.g., methanol, ethanol, etc.], acetone, dioxane, acetonitrile, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely affect the reaction. These conventional solvents may also be used in a mixture with water.

In this reaction, when the compound [III] is used in free acid form or its salt form, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide; N-cyclohexyl-N'-morpholinoethylcarbodiimide; N-cyclohexyl-N'-(4-diethylaminocyclohexyl)carbodiimide; N,N'-diethylcarbodiimide; N,N'-diisopropylcarbodiimide; N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; N,N'-carbonyl-bis-(2-methylimidazole); pentamethyleneketene-N-cyclohexylimine; diphenylketene-N-cyclohexylimine; ethoxyacetylene; 1-alkoxy-1-chloroethylene; trialkyl phosphite; ethyl polyphosphate; isopropyl polyphosphate; phosphorus oxychloride (phosphoryl chloride); phosphorus trichloride; thionyl chloride; oxalyl chloride; lower

alkyl haloformate [e.g., ethyl chloroformate, isopropyl chloroformate, etc.], triphenylphosphine; 2-ethyl-7-hydroxybenzisoxazolium salt; 2-ethyl-5-(m-sulfophenyl)isoxazolium hydroxide intramolecular salt; 1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-benzotriazole; so-called Vilsmeier reagent prepared by the reaction of N,N-dimethylformamide with thionyl chloride, phosgene, trichloromethyl chloroformate, phosphorus oxychloride, etc.; and the like.

The reaction may also be carried out in the presence of an inorganic or organic base such as an alkali metal bicarbonate, tri(lower)alkylamine, pyridine, N-(lower)alkylmorpholine, N,N-di(lower)alkylbenzylamine, and the like.

The reaction temperature is not critical, and the reaction is usually carried out under cooling to warming.

Process 2

The compound [Ib] or a salt thereof can be prepared by subjecting the compound [Ia] or a salt thereof to elimination reaction of the amino protecting group.

Elimination reaction is carried out in accordance with a conventional method such as hydrolysis and the like.

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base includes an inorganic base and an organic base such as an alkali metal [e.g., sodium, potassium, etc.], an alkaline earth metal [e.g., magnesium, calcium, etc.], the hydroxide or carbonate or hydrogencarbonate thereof, trialkylamine [e.g., trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and the like.

Suitable acid includes an organic acid [e.g., formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], and

an inorganic acid [e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g., trichloroacetic acid, trifluoroacetic acid, etc.], and the like is preferably carried out in the presence of cation trapping agents [e.g., anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, alcohol [e.g., methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as a solvent.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

Process 3-(i)

The compound [VIII] or a salt thereof can be prepared by reacting the compound [VI] or a salt thereof with the compound [VII] or a salt thereof.

Suitable salt of the compounds [VI], [VII] and [VIII] can be referred to the ones as exemplified for the compound [I].

The present reaction may be carried out in a solvent such as water, phosphate buffer, acetone, chloroform, acetonitrile nitrobenzene, methylene chloride, ethylene chloride, formamide, N,N-dimethylformamide, methanol, ethanol, diethyl ether, tetrahydrofuran, dimethyl sulfoxide, or any other organic solvent which does not adversely affect the reaction, preferably in ones having strong polarities. Among the solvents, hydrophilic solvents may be used in a mixture with water. When the compound [VII] is liquid, it can also be used as a solvent.

The reaction is preferably conducted in the presence of a base, for example, an inorganic base such as alkali metal hydroxide, alkali metal carbonate, alkali metal hydrogencarbonate, an organic base such as

trialkylamine, and the like.

The reaction temperature is not critical, and the reaction is usually carried out at ambient temperature, under warming or under heating. The present reaction is preferably carried out in the presence of alkali metal halide [e.g., sodium iodide, potassium iodide, etc.], alkali metal thiocyanate [e.g., sodium thiocyanate, potassium thiocyanate, etc.], and the like.

Anion $Z^{\ominus}$ may be one derived from a leaving group Y, and it may be converted to other anion by a conventional method.

Process 3-(ii)

The compound [I] or a salt thereof can be prepared by subjecting the compound [VIII] or a salt thereof to elimination reaction of the carboxy protecting group.

Elimination reaction is carried out in similar manner to the reaction in the aforementioned Process 2, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 2.

Process 4

The compound [Id] or a salt thereof can be prepared by subjecting the compound [Ic] or a salt thereof to elimination reaction of the hydroxy protecting group.

Suitable method of this elimination reaction includes conventional one such as hydrolysis, reduction and the like.

(i) For hydrolysis:

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base includes an inorganic base and an organic base such as an alkali metal [e.g., sodium, potassium, etc.], an alkaline earth metal [e.g., magnesium, calcium, etc.], the hydroxide or carbonate or hydrogencarbonate thereof, trialkylamine [e.g., trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-

diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and the like.

Suitable acid includes an organic acid [e.g., formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], and an inorganic acid [e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g., trichloroacetic acid, trifluoroacetic acid, etc.] and the like is preferably carried out in the presence of cation trapping agents [e.g., anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, alcohol [e.g., methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as a solvent.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

(ii) For reduction:

Reduction is carried out in a conventional manner, including chemical reduction and catalytic reduction.

Suitable reducing reagents to be used in chemical reduction are a combination of a metal [e.g., tin, zinc, iron, etc.] or metallic compound [e.g., chromium chloride, chromium acetate, etc.] and an organic acid or inorganic acid [e.g., formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g., platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g., spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium

sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g., reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g., reduced cobalt, Raney cobalt, etc.], iron catalysts [e.g., reduced iron, Raney iron, etc.], copper catalysts [e.g., reduced copper, Raney copper, Ullman copper, etc.] and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide or a mixture thereof.

Additionally, in case that the above-mentioned acids to be used in chemical reduction are liquid, they can also be used as a solvent.

Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc., or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling to warming.

When R^5 is protected amino, the amino protecting group in R^5 can be eliminated by a conventional method such as hydrolysis.

Processes A and B for the preparation of the starting compounds are explained in detail in the following.

Process A-(i)

The compound [XII] or a salt thereof can be prepared by reacting the compound [X] or a salt thereof with the compound [XI] or a salt thereof.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Process 3-(i), and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 3-(i).

Process A-(ii)

The compound [II] or a salt thereof can be prepared by subjecting the compound [XII] or a salt

thereof to elimination reaction of the amino protecting groups in R^8 and R^{10} and the carboxy protecting group in R^9.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Process 2, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 2.

Process B

The compound [VI] or a salt thereof can be prepared by reacting the compound [XIII] or its reactive derivative at the amino group, or a salt thereof with the compound [XIV] or its reactive derivative at the carboxy group, or a salt thereof.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Process 1, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 1.

The compounds obtained by the above processes can be isolated and purified by a conventional method such as pulverization, recrystallization, column chromatography, reprecipitation, and the like.

It is to be noted that the compound [I] and other compounds may include one or more stereoisomer(s) such as optical isomer(s) and geometrical isomer(s) due to asymmetric carbon atom(s) and double bond(s), and all of such isomers and mixtures thereof are included within the scope of this invention.

The object compounds [I] and pharmaceutically acceptable salts thereof include solvates [e.g., enclosure compounds (e.g., hydrate, etc.)].

The object compound [I] and pharmaceutically acceptable salts thereof are novel and exhibit high antimicrobial activity, inhibiting the growth of a wide variety of pathogenic microorganisms including Gram-positive and Gram-negative microorganisms and are useful as antimicrobial agents.

Now in order to show the utility of the object compound [I], the test data on MIC (minimal inhibitory concentration) of a representative compound of this invention are shown in the following.

Test method:

In vitro antibacterial activity was determined by the two-fold agar-plate dilution method as described below.

One loopful of an overnight culture of each test strain in Trypticase-soy broth (10^6 viable cells per ml) was streaked on heart infusion agar (HI-agar) containing graded concentrations of representative test compound, and the minimal inhibitory concentration (MIC) was expressed in μg/ml after incubation at 37°C for 20 hours.

Test compound

Compound (a): 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(3-aminopropionamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (Example 3)

Compound (b): 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionamido)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (Example 4)

Compound (c): 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate sulfate (Example 6)

Compound (d): 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate sulfate (Example 7)

Compound (e): 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate sulfate (Example 11)

Ceftazidime

Test results:

Table 1

Test strain	Test compound	MIC (μg/ml)
Pseudomonas aeruginosa FP 1380	(a)	2
	(b)	1
	(c)	2
	(d)	2
	(e)	1
	Ceftazidime	128

For therapeutic administration, the object compound [I] and pharmaceutically acceptable salts thereof of the present invention are used in the form of a conventional pharmaceutical preparation which contains said compound as an active ingredient, in admixture with pharmaceutically acceptable carriers such as an organic or inorganic solid or liquid excipient which is suitable for oral, parenteral or external administration. The pharmaceutical preparations may be in a solid form such as tablet, granule, powder, capsule, or in a liquid form such as solution, suspension, syrup, emulsion, lemonade and the like.

If needed, there may be included in the above preparations auxiliary substances, stabilizing agents, wetting agents and other commonly used additives such as lactose, citric acid, tartaric acid, stearic acid, magnesium stearate, terra alba, sucrose, corn starch, talc, gelatin, agar, pectin, peanut oil, olive oil, cacao butter, ethylene glycol, and the like.

While the dosage of the compound [I] may very from and also depend upon the age, conditions of the patient, a kind of diseases, a kind of the compound [I] to be applied, etc. In general amounts between 1 mg and 4,000 mg or even more per day may be administered to a patient. An average single dose of about 50 mg, 100 mg, 250 mg, 500 mg, 1000 mg or 2000 mg of the object compounds [I] of the present invention may be used in treating diseases infected by pathogenic microorganisms.

The following Preparations and Examples are given

for the purpose of illustrating the present invention in more detail.

Preparation 1

To a solution of (Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)[(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (5 g) in a mixture of tetrahydrofuran (80 ml) and N,N-dimethylformamide (20 ml) was added a solution of sodium bis(trimethylsilyl)amide (8.33 g) in tetrahydrofuran (12 ml), and the mixture was stirred for 15 minutes. To the reaction mixture was added a solution of di-tert-butyl dicarbonate (3.3 g) in tetrahydrofuran (20 ml) under ice-cooling, and the mixture was stirred under ice-cooling for 3 hours. To the reaction mixture was added ethyl acetate, and the mixture was washed with 10% aqueous potassium hydrogen sulfate solution, and then washed with a phosphate buffer (pH 6.86). The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether and dried in vacuo to give (Z)-2-{5-[(tert-butoxycarbonyl)amino]-1,2,4-thiadiazol-3-yl}[(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (3.10 g).

IR(KBr) 3191.6, 2981.4, 1714.4, 1550.5, 1153.2, 1000.9 cm^{-1}

^{1}H-NMR(DMSO-d_6) δ 1.37 (9H, s), 1.45 (6H, s), 1.50 (9H, s), 12.7 (1H, s)

ESI-MS: m/z=429(M-H)

Preparation 2

A mixture of N,N-dimethylformamide (0.648 ml) and phosphoryl chloride (0.781 ml) was stirred at room temperature for 30 minutes. To the mixture were added tetrahydrofuran (4 ml) and (Z)-2-{5-[(tert-butoxycarbonyl)amino]-1,2,4-thiadiazol-3-yl}[(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (3 g) at 4°C, and the reaction mixture was stirred at room temperature for 1 hour. Meanwhile, a mixture of benzhydryl 7β-amino-3-chloromethyl-3-cephem-4-

carboxylate hydrochloride (3 g) and N-trimethylsilylacetamide (8.72 g) in tetrahydrofuran (15 ml) was warmed to make a clear solution. The solution was then cooled to -20°C and added to the activated acid solution obtained above. The reaction mixture was stirred at a temperature of -10°C to 0°C for 1 hour and poured into a mixture of ethyl acetate and water. The aqueous layer was separated, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel eluting with hexane/ethyl acetate (3:2) to give benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (4.79 g).

IR(KBr) 2981.4, 1793.5, 1720.2, 1524.8, 1371.1, 1247.7, 1151.3 cm^{-1}

^{1}H-NMR(DMSO-d_6) δ 1.39 (6H, s), 1.48 (3H, s), 1.50 (6H, s), 3.58 (1H, d, J=18.3Hz), 3.76 (1H, d, J=18.3Hz), 4.44 (2H, s), 5.29 (1H, d, J=5.0Hz), 6.01 (1H, dd, J=8.6, 5.0Hz), 6.97 (1H, s), 7.2-7.6 (10H, m), 9.65 (1H, d, J=5.0Hz), 12.7 (1H, s)

ESI-MS: m/z=849(M+Na)

Preparation 3

To a solution of 5-amino-1-methylpyrazole (5 g) in ethanol (50 ml) was added isoamyl nitrite (6.92 ml) and then 20% hydrochloric acid (5 drops) was added at 4°C. The reaction mixture was refluxed for 2 hours and cooled to room temperature. To the reaction mixture was added diisopropyl ether (50 ml) and the mixture was stirred for 0.5 hour. The resulting precipitate was collected by filtration and dried in vacuo to give 5-amino-1-methyl-4-nitrosopyrazole (3.53 g, yield 54.4 %).

^{1}H-NMR(DMSO-d_6) δ 3.51 (3H, s), 8.07 (2H, brs), 8.51 (1H, s)

AP-MS: m/z=127(M+H)

Preparation 4

To a solution of 5-amino-1-methyl-4-nitrosopyrazole (1 g) in water (40 ml) were added concentrated sulfuric acid (0.423 ml) and palladium on carbon (0.3 g) under a hydrogen atmosphere. The mixture was stirred overnight. The reaction mixture was filtered and the filtrate was evaporated in vacuo. To the residue was added isopropyl alcohol and the resulting precipitate was collected by filtration to give 4,5-diamino-1-methylpyrazole sulfuric acid salt (1.71 g, quantitative yield).

^{1}H-NMR(DMSO-d_6) δ 3.54 (3H, s), 7.19 (1H, s)

ESI-MS: m/z=113(M+H)

Preparation 5

To a suspension of 1,1'-carbonyldiimidazole (9.73 g, 60 mmol) in dehydrated chloroform (72 ml) was added tert-butyl N-(2-aminoethyl)carbamate (9.61 g, 60 mmol) under ice-cooling, and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyldiisopropylamine (14.22 g, 110 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (10.51 g, 50 mmol), and the mixture was stirred at 50°C for 15 hours. The insoluble materials were removed by filtration. To the filtrate were added chloroform (200 ml) and 5% aqueous sodium hydrogen carbonate solution (100 ml). The organic layer was separated and the aqueous layer was extracted with a mixed solvent of chloroform and methanol (4:1). The organic layers were combined, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with ethyl acetate and dried in vacuo to give 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}-ureido)-1-methylpyrazole (14.0 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 2.96-2.98 (2H, m), 3.03-3.07 (2H, m), 3.50 (3H, s), 4.81 (2H, br), 5.92 (1H, br), 6.80 (1H, br), 6.96 (1H, s), 7.18 (1H, br)

Example 1

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-

butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (500 mg, 0.60 mmol) in N,N-dimethylformamide (1.0 ml) was added sodium iodide (100 mg, 0.66 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added a solution of 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-1-methylpyrazole (216 mg, 0.73 mmol) in N,N-dimethylformamide (1.0 ml). The whole mixture was stirred at 32°C for 4 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (75 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (1.8 ml) were added anisole (0.6 ml) and trifluoroacetic acid (1.2 ml). The resulting solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (80 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (380 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (21 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 3.12 (2H, t,

J=5.7Hz), 3.22 (1H, d, J=17.9Hz), 3.49 (1H, d, J=17.9Hz), 3.46 (2H, t, J=5.7Hz), 3.71 (3H, s), 4.95 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.6Hz), 5.84 (1H, d, J=4.6Hz), 7.89 (1H, s)

Preparation 6

To a solution of 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-1-methylpyrazole (597 mg, 2 mmol) and triethylamine (243 mg, 2.4 mmol) in methylene chloride (10 ml) was added triphenylmethyl chloride (669 mg, 2.4 mmol), and the mixture was stirred at room temperature for 19 hours. The reaction mixture was washed successively with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with ethyl acetate to give 4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}-ureido)-1-methyl-5-triphenylmethylaminopyrazole (640 mg) as a solid.

^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 2.70 (3H, s), 2.94-2.96 (2H, m), 2.99-3.01 (2H, m), 5.68 (1H, brs), 5.96 (1H, br), 6.78 (1H, br), 6.85 (1H, br), 7.00 (1H, s), 7.13-7.15 (6H, m), 7.24-7.28 (9H, m)

Example 2

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (810 mg, 1.0 mmol) in N,N-dimethylformamide (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added a solution of 4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-1-methyl-5-triphenylmethylaminopyrazole (640 mg, 1.2 mmol) in methylene chloride (10 ml). The whole mixture was stirred at room temperature for 26 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated,

and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (80 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (2.38 ml) were added anisole (0.79 ml) and trifluoroacetic acid (1.58 ml). The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether (80 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (635 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (54 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 3.12 (2H, t, J=5.7Hz), 3.22 (1H, d, J=17.9Hz), 3.49 (1H, d, J=17.9Hz), 3.46 (2H, t, J=5.7Hz), 3.71 (3H, s), 4.95 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.6Hz), 5.84 (1H, d, J=4.6Hz), 7.89 (1H, s)

Preparation 7

To a solution of 2,3-dihydro-1H-imidazo[1,2-b]pyrazole (120 g, 1.1 mol) in sulfuric acid (500 ml) was added potassium nitrate (111 g, 1.1 mol) under ice-cooling. The mixture was stirred at room temperature for 48 hours. The reaction mixture was added to ice (2.0 kg). The crystalline residue was collected by

filtration and dried in vacuo to give 7-nitro-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (132 g) as a solid.
^{1}H-NMR(DMSO-d_6) δ 4.05-4.09 (2H, m), 4.17-4.20 (2H, m), 7.82 (1H, s), 7.97 (1H, br)

Preparation 8

A suspension of 7-nitro-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (97 g, 629 mmol) in a mixed solvent of sulfuric acid (34 ml) and water (2000 ml) was treated with 10% palladium on carbon (10 g) under a hydrogen atmosphere at room temperature for 4 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with methanol and dried in vacuo to give 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (90.2 g) as a solid.
^{1}H-NMR(DMSO-d_6) δ 3.87-3.90 (2H, m), 4.07-4.10 (2H, m), 7.28 (1H, s)

Preparation 9

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (2.22 g, 10 mmol) and N-ethyldiisopropylamine (2.84 g, 22 mmol) in methylene chloride (70 ml) was added N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (3.15 g, 11 mmol). The mixture was stirred at room temperature for 4 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution and the organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 7-[3-(tert-butoxycarbonylamino)propionyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (2.2 g) as a solid.
^{1}H-NMR($CDCl_3$) δ 1.44 (9H, s), 2.52 (2H, t, J=6.0Hz), 3.36-3.47 (2H, m), 3.96 (2H, t, J=8.2Hz), 4.18 (2H, t, J=8.2Hz), 5.16 (1H, br), 7.16 (1H, s), 7.90 (1H, br)

Example 3

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(3-

aminopropionamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 7-[3-(tert-butoxycarbonylamino)propionyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole in the same manner as in Example 1 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (3H, s), 1.52 (3H, s), 2.83 (2H, t, J=6.4Hz), 3.26 (1H, d, J=17.9Hz), 3.53 (1H, d, J=17.9Hz), 3.31 (2H, t, J=6.4Hz), 4.15 (2H, t, J=8.7Hz), 4.33 (1H, q, J=8.7Hz), 4.42 (1H, q, J=8.7Hz), 4.95 (1H, d, J=15.1Hz), 5.03 (1H, d, J=15.1Hz), 5.25 (1H, d, J=5.0Hz), 5.84 (1H, d, J=5.0Hz), 8.06 (1H, s)

Preparation 10

tert-Butyl [2-(5-amino-1-methyl-1H-pyrazol-4-ylcarbamoyl)ethyl]carbamate

The title compound was obtained from 4,5-diamino-1-methylpyrazole sulfuric acid salt and N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide in the same manner as in Preparation 9.

^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 2.35 (2H, t, J=7.1Hz), 3.18 (2H, q, J=7.1Hz), 3.50 (3H, s), 4.90 (2H, s), 6.83 (1H, t, J=7.1Hz), 7.14 (1H, s), 9.06 (1H, s)

AP-MS: m/z=283

Preparation 11

tert-Butyl {2-[1-methyl-5-(tritylamino)-1H-pyrazol-4-ylcarbamoyl]ethyl}carbamate

The title compound was obtained from tert-butyl [2-(5-amino-1-methyl-1H-pyrazol-4-ylcarbamoyl)ethyl]-carbamate in the same manner as in Preparation 6.

^{1}H-NMR(DMSO-d_6) δ 1.39 (9H, s), 2.08 (2H, t, J=7.1Hz), 2.71 (3H, s), 3.04 (2H, q, J=7.1Hz), 5.57 (1H, s), 6.72 (1H, t, J=7.1Hz), 7.1-7.4 (16H, m), 8.25 (1H, s)

Example 4

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-

aminopropionamido)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and tert-butyl (2-([1-methyl-5-(tritylamino)-1H-pyrazol-4-ylcarbamoyl]ethyl)carbamate in the same manner as in Example 1.

^{1}H-NMR(D_2O) δ 1.53 (6H, s), 2.89 (2H, t, J=6.5Hz), 3.20 and 3.47 (2H, ABq, J=18Hz), 3.34 (2H, t, J=6.5Hz), 3.75 (3H, s), 4.99 and 5.21 (2H, ABq, J=16Hz), 5.25 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.02 (1H, s)

ESI-MS: m/z=674(M+Na)

Preparation 12

To a solution of 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethylsulfonyl)guanidine (22.3 g) in dichloromethane (100 ml) were added 4,5-diamino-1-methylpyrazole sulfuric acid salt (10 g) and triethylamine (33.2 ml) at 4°C, and the mixture was stirred at room temperature overnight. The reaction mixture was poured into a mixture of ethyl acetate and water. The aqueous layer was separated, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo. The concentrate was poured into acetonitrile, and the resulting precipitate was collected by filtration and dried in vacuo to give 5-amino-4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methylpyrazole (11.62 g, yield 68.9%).

^{1}H-NMR(DMSO-d_6) δ 1.37 (9H, s), 1.50 (9H, s), 3.52 (3H, s), 5.14 (2H, s), 7.11 (1H, s), 9.14 (1H, s), 11.5 (1H, s)

ESI-MS: m/z=353(M-H)

Preparation 13

4-[2',3'-Bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-(tritylamino)pyrazole

The title compound was obtained from 5-amino-4-

[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methylpyrazole in the same manner as in Preparation 6.
^{1}H-NMR(DMSO-d_6) δ 1.37 (9H, s), 1.50 (9H, s), 2.85 (3H, s), 5.88 (1H, s), 7.17 (1H, s), 7.21 (15H, m), 8.85 (1H, s), 11.2 (1H, s)
ESI-MS: m/z=597(M+H)

Example 5

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-(tritylamino)pyrazole in the same manner as in Example 1.
^{1}H-NMR(DMSO-d_6) δ 1.53 (6H, s), 3.25 and 3.57 (2H, ABq, J=18Hz), 3.75 (3H, s), 5.00 and 5.18 (2H, ABq, J=15Hz), 5.27 (1H, d, J=4.9Hz), 5.85 (1H, d, J=4.9Hz), 8.05 (1H, s)

Preparation 14

To a suspension of 4,5-diamino-1-methylpyrazole sulfuric acid salt (305 g, 1.45 mol) in tetrahydrofuran (3.05 L) was added tert-butyl 2-[(2,5-dioxo-1-pyrrolidinyl)oxy]-2-oxoethylcarbamate (415 g, 1.52 mol) under ice-cooling. To the mixture was added diisopropylethylamine (556 ml, 3.19 mol) dropwise at a temperature below 10°C. The mixture was stirred at room temperature overnight. To the resulting solution were added brine and saturated aqueous sodium hydrogen carbonate solution, and the mixture was extracted with ethyl acetate (3.0 L). The aqueous layer was extracted with tetrahydrofuran/ethyl acetate=1/1 (3.0 L) twice. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether (1.0 L) and dried in vacuo to give tert-butyl 2-[(5-amino-1-

methyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate (307 g).

IR(KBr) 3440, 3349, 1670, 1631, 1525, 1276, 1163, 1074, 1014, 860, 791 cm^{-1}

^{1}H-NMR(DMSO-d_6) δ 1.39 (9H, s), 3.44 (3H, s), 3.64 (2H, d, J=5.9Hz), 4.91 (2H, brs), 6.97 (1H, t, J=5.9Hz), 7.15 (1H, s), 9.09 (1H, brs)

Preparation 15

To a solution of tert-butyl 2-[(5-amino-1-methyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate (307 g, 1.14 mol) in N,N-dimethylformamide (1.5 L) was added triphenylmethyl chloride (334 g, 1.2 mol). To the mixture was added triethylamine (318 ml, 2.28 mol) dropwise. The mixture was stirred at room temperature for 1 hour. The reaction mixture was dissolved in ethyl acetate. The solution was washed successively with water, 10% aqueous citric acid solution, water, and brine. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with acetonitrile (1.5 L) and dried in vacuo to give tert-butyl 2-({[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}-2-oxoethylcarbamate (468 g).

IR(KBr) 3336, 3280, 1724, 1683, 1599, 1234, 939, 704 cm^{-1}

^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 2.73 (3H, s), 3.38 (2H, d, J=5.8Hz), 5.58 (1H, s), 6.94 (1H, t, J=5.8Hz), 7.11-7.35 (15H, m), 7.21 (1H, s), 8.31 (1H, s)

ESI-MS: m/z=512.3($M+H^+$)

Example 6

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (489 g) in N,N-dimethylformamide (1.4 L) was added sodium iodide (102 g). After stirring at room temperature for 1 hour, tert-butyl 2-({[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}-2-oxoethylcarbamate (383 g) was added to the mixture. Stirring was

continued at 37°C for 24 hours. The resulting mixture was poured into water and extracted with ethyl acetate. The organic layer was washed successively with water, 10% aqueous sodium thiosulfate solution, brine and 10% aqueous sodium trifluoroacetate solution, and dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was dissolved in ethyl acetate (3.5 L), and the solution was dropwise added to diisopropyl ether (36 L). The precipitate was collected by filtration. The filter cake was washed with diisopropyl ether and dried in vacuo.

The obtained solid (700 g) was dissolved in dichloromethane (1.4 L) and to the solution were added anisole (700 ml) and trifluoroacetic acid (2.1 L) successively. After stirring at room temperature for 4 hours, the reaction mixture was poured into diisopropyl ether (30 L). The precipitate was collected by filtration. The obtained solid was washed with diisopropyl ether and dried in vacuo. The crude product was dissolved in water (3.5 L), and the pH of the solution was adjusted to 7.0 with 28% aqueous ammonia solution. The insoluble material was filtered off, and the pH of the filtrate was adjusted to 1 with concentrated hydrochloric acid, and the insoluble material was filtered off again. The filtrate was chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.0 L in vacuo and 2.0M sulfuric acid (102 ml) was added to the concentrate. The mixture was freeze-dried to give the crude product.

The crude product was purified by preparative HPLC (pH 7.0 phosphate buffer and acetonitrile), and the eluate containing a desired product was concentrated to about 6 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 550 ml in vacuo and 2.0M sulfuric acid (54.5 ml)

was added to the concentrate. To the mixture was added dropwise acetonitrile (880 ml) and the mixture was stirred at room temperature overnight. To the mixture was added acetonitrile (200 ml) and the mixture was stirred at room temperature for 2 hours. The resulting white crystals were collected by filtration and washed with 25% aqueous acetonitrile and dried under reduced pressure to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate sulfate (72.5 g).

IR(KBr) 1778, 1700, 1653, 1525, 1149, 1111, 617 cm^{-1}

^{1}H-NMR(D_2O) δ 1.61 (6H, s), 3.22 and 3.45 (2H, ABq, J=17.8Hz), 3.73 (3H, s), 4.03 (2H, s), 5.05 and 5.27 (2H, ABq, J=15.8Hz), 5.25 (1H, d, J=4.8Hz), 5.87 (1H, d, J=4.8Hz), 8.09 (1H, s)

ESI-MS: m/z=638.2($M+H^+$)

Example 7

A solution of 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (36 g) in water was purified by preparative HPLC utilizing ODS column. The eluate containing a desired product was concentrated to about 1.5 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (6 L) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 800 ml in vacuo and 2M sulfuric acid (17 ml) was added. The resulting solution was lyophilized to give a sulfuric acid salt as an amorphous powder (23.6 g).

The powder was dissolved in water (71 ml) and ethanol (57 ml). After addition of seed crystals (310 mg), which resulted in the precipitation of white solid, the mixture was stirred for 1 hour. A mixture of ethanol (47 ml) and water (37 ml) was added over 30 minutes and ethanol (33 ml) was added over 20 minutes. Then the slurry was stirred for an additional 1.5 hour.

The precipitate was collected by filtration, washed with ethanol/water (60 ml/20 ml) and ethanol (60 ml) and dried to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate sulfate as crystals (17.3 g).

IR(KBr) 3353, 3183, 1778, 1652, 1558, 1403, 1321, 1143, 1118, 997, 619 cm^{-1}

^{1}H-NMR(D_2O) δ 1.61 (6H, s), 3.10-3.55 (6H, m), 3.71 (3H, s), 5.02 and 5.23 (2H, ABq, J=16.7Hz), 5.25 (1H, d, J=4.9Hz), 5.87 (1H, d, J=4.9Hz), 7.91 (1H, s)

ESI-MS: m/z=667($M+H^+$)

X-ray powder diffraction analysis (by Rigaku X-ray Diffraction system MultiFlex)

2θ	intensity
8.0	1286
12.7	586
13.8	423
16.1	618
18.9	520
20.4	748
21.5	667
22.4	1058
23.3	944
24.0	618
25.5	813
26.7	472
27.9	537
28.5	455
31.3	390

X-ray: Cu/40 kV/30 mA

Preparation 16

5-Amino-1-ethyl-4-nitrosopyrazole

The title compound was obtained from 5-amino-1-ethylpyrazole in the same manner as in Preparation 3.

^{1}H-NMR(DMSO-d_6) δ 1.21 (3H, t, J=7.1Hz), 3.93 (2H, q, J=7.1Hz), 7.04 and 8.53 (1H, s), 8.10 and 8.15 (1H, brs)

APCI-MS: m/z=141($M+H$)$^+$

Preparation 17

4,5-Diamino-1-ethylpyrazole sulfuric acid salt

The title compound was obtained from 5-amino-1-ethyl-4-nitrosopyrazole in the same manner as in Preparation 4.

^{1}H-NMR(D_2O) δ 1.36 (3H, t, J=7.3Hz), 4.10 (2H, q, J=7.3Hz), 7.77 (1H, s)

ESI-MS: m/z=127$(M+H)^+$

Preparation 18

5-Amino-4-[3-(tert-butoxycarbonylamino)-propionylamino]-1-ethylpyrazole

The title compound was obtained from 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 14.

^{1}H-NMR(DMSO-d_6) δ 1.24 (3H, t, J=7.2Hz), 1.37 (9H, s), 2.35 (2H, t, J=7.1Hz), 3.18 (2H, dt, J=7.1Hz, 7.1Hz), 3.85 (q, J=7.2Hz), 4.88 (2H, brs), 6.75-6.90 (1H, m), 7.17 (1H, s), 9.05 (1H, brs)

APCI-MS: m/z=298$(M+H)^+$

Preparation 19

4-[3-(tert-Butoxycarbonylamino)propionylamino]-1-ethyl-5-triphenylmethylaminopyrazole

The title compound was obtained from 5-amino-4-[3-(tert-butoxycarbonylamino)propionylamino]-1-ethylpyrazole in the same manner as in Preparation 15.

^{1}H-NMR(DMSO-d_6) δ 0.88 (3H, t, J=7.2Hz), 1.39 (9H, s), 2.02 (2H ,t, J=7.1Hz), 2.95-3.20 (4H, m), 5.59 (1H, brs), 6.60-6.75 (1H, m), 7.10-7.35 (16H, m), 8.04 (1H, brs)

ESI-MS: m/z=540$(M+H)^+$, 562$(M+Na)^+$

Example 8

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionylamino)-2-ethyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-[3-(tert-

butoxycarbonylamino)propionylamino]-1-ethyl-5-triphenylmethylaminopyrazole in the same manner as in Example 1.

IR(KBr) 3415, 1763, 1658, 1598, 1529, 1402, 1361 cm^{-1}

^1H-NMR(D_2O) δ 1.33 (3H, t, J=7.2Hz), 1.53 (6H, s), 2.89 (2H, t, J=6.5Hz), 3.17 and 3.49 (2H, ABq, J=17.7Hz), 3.34 (2H, t, J=6.5Hz), 4.28 (2H, q, J=7.2Hz), 5.05 and 5.16 (2H, ABq, J=15.4Hz), 5.26 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.03 (1H, s)

Preparation 20

tert-Butyl 2-[(5-amino-1-ethyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate

The title compound was obtained from 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 14.

^1H-NMR(DMSO-d_6) δ 1.21 (3H, t, J=7.2Hz), 1.39 (9H, s), 3.64 (2H, d, J=6.0Hz), 3.86 (2H, d, J=7.2Hz), 4.88 (2H, brs), 6.90-7.00 (1H, m), 7.17 (1H, s), 9.06 (1H, brs)

ESI-MS: m/z=284 $(M+H)^+$, 306 $(M+Na)^+$

Preparation 21

tert-Butyl 2-{[1-ethyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}-2-oxoethylcarbamate

The title compound was obtained from tert-butyl 2-[(5-amino-1-ethyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate in the same manner as in Preparation 15.

^1H-NMR(DMSO-d_6) δ 0.88 (3H, t, J=7.2Hz), 1.38 (9H, s), 3.16 (2H, q, J=7.2Hz), 3.31 (2H, d), 5.59 (1H, brs), 6.80-6.95 (1H, m), 7.10-7.40 (16H, m), 8.03 (1H, brs)

ESI-MS: m/z=526 $(M+H)^+$, 548 $(M+Na)^+$

Example 9

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-ethyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-

iodomethyl-3-cephem-4-carboxylate and tert-butyl 2-{[1-ethyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}-2-oxoethylcarbamate in the same manner as in Example 1.
IR(KBr) 3444, 1761, 1635, 1626, 1446, 1406 cm^{-1}
^{1}H-NMR(D_2O) δ 1.33 (3H, t, J=7.2Hz), 1.53 (6H, s), 2.89 (2H, t, J=6.5Hz), 3.17 and 3.49 (2H, ABq, J=17.7Hz), 4.00 (2H, s), 4.28 (2H, q, J=7.2Hz), 5.06 and 5.17 (2H, ABq, J=15.4Hz), 5.27 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.07 (1H, s)

Preparation 22

5-Amino-4-[2',3'-bis(tert-butoxycarbonyl)-guanidino]-1-ethylpyrazole

The title compound was obtained from 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethylsulfonyl)guanidine and 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 12.
^{1}H-NMR(DMSO-d_6) δ 1.22 (3H, t, J=7.1Hz), 1.37 (9H, s), 1.50 (9H, s), 3.88 (2H, d, J=7.1Hz), 5.12 (2H, brs), 7.14 (1H, s), 9.16 (1H, brs), 11.51 (1H, brs)
ESI-MS: m/z=369 $(M+H)^+$

Preparation 23

4-[2',3'-Bis(tert-butoxycarbonyl)guanidino]-1-ethyl-5-triphenylmethylaminopyrazole

The title compound was obtained from 5-amino-4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-ethylpyrazole in the same manner as in Preparation 15.
^{1}H-NMR(DMSO-d_6) δ 0.86 (3H, t, J=7.1Hz), 1.38 (9H, s), 1.49 (9H, s), 5.85 (1H, brs), 7.10-7.30 (16H, m), 8.80 (1H, brs), 11.14 (1H, brs)
ESI-MS: m/z=611 $(M+H)^+$, 633 $(M+Na)^+$

Example 10

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-2-ethyl-4-guanidino-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-

iodomethyl-3-cephem-4-carboxylate and 4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-ethyl-5-triphenylmethylaminopyrazole in the same manner as in Example 1.

IR(KBr) 3437, 1760, 1658, 1625, 1406, 1065 cm^{-1}

^1H-NMR(D_2O) δ 1.35 (3H, t, J=7.3Hz), 1.53 (6H, s), 3.26 and 3.61 (2H, ABq, J=17.8Hz), 4.29 (2H, q, J=7.3Hz), 5.06 and 5.17 (2H, ABq, J=15.7Hz), 5.29 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.06 (1H, s)

Example 11

To a suspension of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (500 g, 611 mmol) in N,N-dimethylformamide (2.5 L) was added 4-[2',3'-bis(tert-butoxycarbonyl)-guanidino]-1-methyl-5-triphenylmethylaminopyrazole (419 g) and the mixture was stirred at room temperature for 16 hours. The reaction mixture was added to a mixture of ethyl acetate and water. The organic layer was washed with water, brine and 10% aqueous sodium trifluoroacetate solution and then dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated to 3.3 kg under reduced pressure. The concentrate was poured into diisopropyl ether (33 L), and the resulting precipitate was collected by filtration and dried in vacuo.

To a solution of the resulting solid in methylene chloride (1500 ml) were added anisole (500 ml) and trifluoroacetic acid (1500 ml). The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether. The resulting precipitate was collected by filtration and dried in vacuo. The crude product was dissolved in water (3.5 L), and the pH of the solution was adjusted to 7.0 with 28% aqueous ammonia solution. The insoluble material was filtered off, and the pH of the filtrate was adjusted to 1 with concentrated hydrochloric acid, and the insoluble material was filtered off, again. The filtrate was

chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.0 L in vacuo. To the concentrate was added 2.0M sulfuric acid (150 ml) and the mixture was freeze-dried to give the crude product. The crude product was purified with preparative HPLC utilizing ODS column (pH 7.0 phosphate buffer and acetonitrile). The eluate containing a desired product was concentrated to about 6 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 1.5 L in vacuo. To the concentrate was added 2.0M sulfuric acid (60 ml) and the mixture was freeze-dried to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate sulfate (48.5 g, yield 11%).

IR(KBr) 1776, 1714, 1677, 1651, 1402, 1112 cm^{-1}

^1H-NMR(D_2O) δ 1.61 (6H, s), 3.28 and 3.58 (2H, ABq, J=17.8Hz), 3.74 (3H, S), 5.15 and 5.23 (2H, ABq, J=15.7Hz), 5.27 (1H, d, J=4.8Hz), 5.88 (1H, d, J=4.8Hz), 8.07 (1H, s)

ESI-MS: m/z=623.2($M+H^+$)

Preparation 24

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (2.4 g, 10 mmol) in methylene chloride (40 ml) were added N-ethyldiisopropylamine (2.1 ml, 12 mmol) and N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (2.3 g, 8 mmol) under ice-cooling, and the mixture was stirred at room temperature for 6 hours. To the reaction mixture were added brine (40 ml) and saturated aqueous sodium hydrogen carbonate solution (20 ml), and the mixture was extracted with a mixture of ethyl acetate and 2-propanol (3:1, 60 ml). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diethyl ether to

give 5-amino-4-[3-(tert-butoxycarbonylamino)propionyl]amino-1-(2-hydroxyethyl)pyrazole (1.65 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 2.35 (2H, t, J=7.3Hz), 3.16-3.20 (2H, m), 3.62-3.65 (2H, m), 3.90 (2H, t, J=6.0Hz), 4.85 (2H, brs), 4.92 (1H, t, J=5.0Hz), 6.84 (1H, t, J=5.5Hz), 7.20 (1H, s), 9.09 (1H, brs)

Example 12

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionamido)-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[3-(tert-butoxycarbonylamino)propionyl]amino-1-(2-hydroxyethyl)pyrazole in the same manner as in Example 1 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (6H, s), 2.88 (2H, t, J=6.4Hz), 3.15 (1H, d, J=17.9Hz), 3.48 (1H, d, J=17.9Hz), 3.32 (2H, t, J=6.4Hz), 3.88 (2H, t, J=4.8Hz), 4.39 (1H, dt, J=16.5Hz, 4.8Hz), 4.42 (1H, dt, J=16.5Hz, 4.8Hz), 5.06 (1H, d, J=15.1Hz), 5.11 (1H, d, J=15.1Hz), 5.25 (1H, d, J=5.0Hz), 5.83 (1H, d, J=5.0Hz), 8.05 (1H, s)

Preparation 25

To a solution of 4-formyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (1.51 g, 10 mmol) in sulfuric acid (7.5 ml) was added potassium nitrate (111 g, 1.1 mol) under ice-cooling. The mixture was stirred at room temperature for 17 hours. The reaction mixture was added to ice (100 g). The crystalline residue was collected by filtration and dried in vacuo to give 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (0.63 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 2.00-2.05 (2H, m), 3.30-3.36 (2H, m), 3.99 (2H, t, J=6.0Hz), 7.85 (1H, s), 7.89 (1H, s)

Preparation 26

A solution of 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (1.68 g, 10 mmol) in a mixture of sulfuric acid (0.6 ml), acetic acid (100 ml) and water (10 ml) was treated with 10% palladium on carbon (0.5 g) under a hydrogen atmosphere at room temperature for 6 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with ethanol and dried in vacuo to give 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.3 g) as a solid.
^{1}H-NMR(DMSO-d_6) δ 1.97-2.01 (2H, m), 3.22 (2H, t, J=5.0Hz), 3.98 (2H, t, J=6.0Hz), 7.22 (1H, s)

Preparation 27

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.96 g, 10 mmol) and N-ethyldiisopropylamine (3.88 g, 30 mmol) in methylene chloride (70 ml) was added 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)-guanidine (3.91 g, 10 mmol). The mixture was stirred at room temperature for 150 minutes. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 2% methanol/chloroform to give 3-[2,3-bis(tert-butoxycarbonyl)guanidino]-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (3.4 g) as a solid.
^{1}H-NMR($CDCl_3$) δ 1.48 (9H, s), 1.52 (9H, s), 2.12-2.14 (2H, m), 3.33-3.37 (2H, m), 4.08 (2H, t, J=6.0Hz), 6.17 (1H, brs), 7.16 (1H, s), 9.87 (1H, brs), 11.39 (1H, brs)

Example 13

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.2 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide (181 mg, 1.2 mmol), and the mixture was stirred at room

temperature for 30 minutes. To the reaction mixture were added 3-[2,3-bis(tert-butoxycarbonyl)guanidino]-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (571 mg, 1.5 mmol) and methylene chloride (2.0 ml). The whole mixture was stirred at room temperature for 7 hours. To the reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (150 ml), and the resulting precipitate was collected by filtration and dried in vacuo.

To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml) and the mixture was stirred at room temperature for 4 hours. The reaction mixture was poured into diisopropyl ether (150 ml) and the resulting precipitate was collected by filtration and dried in vacuo to give a crude product (570 mg), which was purified by preparative HPLC utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-guanidino-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate (51 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 2.05-2.25 (2H, m), 3.26 (1H, d, J=17.4Hz), 3.56 (1H, d, J=17.4Hz), 3.30-3.45 (2H, m), 4.15 (2H, t, J=6.0Hz), 4.93 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 7.99 (1H, s)

Preparation 28

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (4.4 g, 20 mmol), 4-(dimethylamino)pyridine (244 mg, 2 mmol) and triethylamine (8.10 g, 80 mmol) in chloroform (45 ml) was added 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)guanidine (10.18 g, 26 mmol). The mixture was stirred at room temperature for 2 hours. The reaction mixture was washed successively with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether to give 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (4.6 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.49 (9H, s), 1.52 (9H, s), 3.97-4.01 (2H, m), 4.21 (2H, t, J=7.8Hz), 5.30 (1H, brs), 7.19 (1H, s), 9.86 (1H, brs), 11.32 (1H, brs)

Example 14

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-guanidino-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole in the same manner as in Example 13 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (3H, s), 1.52 (3H, s), 3.35 (1H, d, J=17.9Hz), 3.61 (1H, d, J=17.9Hz), 4.19 (2H, t, J=8.7Hz), 4.37 (1H, q, J=8.7Hz), 4.47 (1H, q, J=8.7Hz), 5.00 (1H, d, J=15.1Hz), 5.04 (1H, d, J=15.1Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.13 (1H, s)

Preparation 29

To a solution of 5-amino-1-(2-

hydroxyethyl)pyrazole (6.35 g, 50 mmol) in a mixed solvent of ethanol (25 ml) and concentrated hydrochloric acid (0.035 ml) was added dropwise isoamyl nitrite (7.03 g, 60 mmol). The mixture was stirred at room temperature for 17 hours. The crystalline residue was collected by filtration and dried in vacuo to give 5-amino-1-(2-hydroxyethyl)-4-nitrosopyrazole (4.0 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 3.68 (2H, t, J=5.5Hz), 3.94 (2H, t, J=5.5Hz), 4.89 (1H, br), 8.06(2H, br), 8.53 (1H, s)

Preparation 30

A solution of 5-amino-1-(2-hydroxyethyl)-4-nitrosopyrazole (97 g, 629 mmol) in a mixed solvent of sulfuric acid (34 ml) and water (2000 ml) was treated with 10% palladium on carbon (10 g) under a hydrogen atmosphere at room temperature for 4 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with methanol and dried in vacuo to give 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (90.2 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 3.66 (2H, t, J=5.5Hz), 3.95 (2H, t, J=5.5Hz), 7.25 (1H, s)

Preparation 31

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (50.0 g, 208 mmol) in chloroform (500 ml) were added 4-(dimethylamino)pyridine (2.54 g, 20.8 mmol), triethylamine (116 ml, 833 mmol) and 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)guanidine (106 g, 271 mmol). The mixture was stirred under reflux for 2 hours. After cooling on an ice bath, the reaction mixture was washed successively with water, 4% aqueous citric acid solution, water and aqueous sodium hydrogen carbonate solution. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was triturated with a mixed solvent of ethyl acetate (50 ml) and diethyl ether (200 ml) to give 5-

amino-4-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-(2-hydroxyethyl)pyrazole (50 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.47 (9H, s), 1.53 (9H, s), 3.28 (1H, br), 4.02-4.05 (4H, m), 4.65 (2H, br), 7.22 (1H, s), 9.85 (1H, br), 11.55 (1H, br)

Example 15

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-guanidino-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-(2-hydroxyethyl)pyrazole in the same manner as in Example 13 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 3.21 (1H, d, J=17.9Hz), 3.59 (1H, d, J=17.9Hz), 3.90 (2H, t, J=4.8Hz), 4.35-4.50 (2H, m), 5.07 (1H, d, J=14.9Hz), 5.11 (1H, d, J=14.9Hz), 5.28 (1H, d, J=5.0Hz), 5.84 (1H, d, J=5.0Hz), 8.09 (1H, s)

Preparation 32

To a solution of 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.83 g, 5 mmol) in pyridine (10 ml) was added triphenylmethyl chloride (1.67 g, 6 mmol). The mixture was stirred at 50°C for 5 hours. After cooling, chloroform (50 ml) was added to the reaction mixture, and the mixture was washed successively with 10% aqueous citric acid solution, brine, and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 2% methanol/chloroform to give 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-triphenylmethyl-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.57 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.47 (9H, s), 1.48 (9H, s), 3.50 (2H, t,

J=7.8Hz), 3.92 (2H, t, J=7.8Hz), 7.07-7.26 (10H, m), 7.53-7.54 (6H, m), 8.34 (1H, brs), 11.12 (1H, brs)

Example 16

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (819 mg, 1.0 mmol) in N,N-dimethylformamide (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-triphenylmethyl-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (730 mg, 1.2 mmol). The whole mixture was stirred at room temperature for 6 hours. To the resulting reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution, 10% aqueous sodium thiosulfate solution and brine, dried over sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (120 ml), and the resulting precipitate was collected by filtration and dried in vacuo.

To a solution of the resulting solid in methylene chloride (2.0 ml) were added anisole (0.67 ml) and trifluoroacetic acid (1.34 ml) and the mixture was stirred at room temperature for 4 hours. The reaction mixture was poured into diisopropyl ether (120 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (430 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The

eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-guanidino-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (20.4 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (3H, s), 1.52 (3H, s), 3.35 (1H, d, J=17.9Hz), 3.61 (1H, d, J=17.9Hz), 4.19 (2H, t, J=8.7Hz), 4.37 (1H, q, J=8.7Hz), 4.47 (1H, q, J=8.7Hz), 5.00 (1H, d, J=15.1Hz), 5.04 (1H, d, J=15.1Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.13 (1H, s)

Preparation 33

To a suspension of 1,1'-carbonyldiimidazole (1.94 g, 12 mmol) in methylene chloride (20 ml) was added tert-butyl N-(3-aminopropyl)carbamate (2.30 g, 13.2 mmol), and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyldiisopropylamine (2.56 g, 20 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (2.10 g, 10 mmol), and the mixture was stirred at 30°C for 3 days. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 6% methanol/chloroform to give 5-amino-4-(3-{3-[(tert-butoxycarbonyl)amino]propyl}ureido)-1-methylpyrazole (1.75 g) as a solid.

^{1}H-NMR(DMSO-d_6) δ 1.37 (9H, s), 1.43-1.49 (2H, m), 2.89-2.93 (2H, m), 2.98-3.01 (2H, m), 3.50 (3H, s), 4.79 (2H, br), 5.85 (1H, br), 6.77 (1H, br), 6.96 (1H, s), 7.12 (1H, br)

Example 17

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.21 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide (199 mg, 1.33 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 5-amino-4-(3-{3-[(tert-

butoxycarbonyl)amino]propyl}ureido)-1-methylpyrazole (415 mg, 1.33 mmol) and the whole mixture was stirred at 32°C for 24 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (100 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (3.6 ml) were added anisole (1.2 ml) and trifluoroacetic acid (2.4 ml). The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether (100 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (939 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(3-aminopropyl)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (53 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 1.85-1.88 (2H, m), 3.03 (2H, t, J=8Hz), 3.22(2H, t, J=18Hz), 3.26 (2H, t, J=7Hz), 3.49 (1H, d, J=18Hz), 3.72 (3H, s), 4.96 (1H, d, J=15Hz), 5.16 (1H, d, J=15Hz), 5.25 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 7.88 (1H, s)

Preparation 34

To a suspension of 1,1'-carbonyldiimidazole (973 mg, 6 mmol) in methylene chloride (10 ml) was added

tert-butyl N-(2-aminoethyl)carbamate (1.11 g, 6.9 mmol) under ice-cooling, and the mixture was stirred at room temperature for 2 hours. To the reaction mixture were added N-ethyldiisopropylamine (1.28 g, 10 mmol) and 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (1.18 g, 5 mmol), and the mixture was stirred at 50°C for 6 hours. The reaction mixture was washed with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 3-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (150 mg) as a solid.
^{1}H-NMR($CDCl_3$) δ 1.43 (9H, s), 2.11-2.16 (2H, m), 3.22-3.35 (6H, m), 4.09 (2H, t, J=7Hz), 4.69 (1H, br), 5.14 (2H, br), 5.69 (1H, br), 7.17 (1H, s)

Example 18

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-[3-(2-aminoethyl)ureido]-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine in the same manner as in Example 17 as an amorphous solid.
^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 2.09-2.21 (2H, m), 3.13 (2H, t, J=6Hz), 3.24 (1H, d, J=18Hz), 3.35-3.52 (5H, m), 4.12-4.15 (2H, m), 4.88 (1H, d, J=16Hz), 5.13 (1H, d, J=16Hz), 5.25 (1H, d, J=5Hz), 5.85 (1H, d, J=5Hz), 7.83 (1H, s)

Preparation 35

To a suspension of 1,1'-carbonyldiimidazole (973 mg, 6 mmol) in methylene chloride (10 ml) was added O-[2-(tert-butoxycarbonylamino)ethyl]hydroxylamine (1.11 g,

6.3 mmol) under ice-cooling, and the mixture was stirred at room temperature for 2 hours. To the reaction mixture were added N-ethyldiisopropylamine (1.28 g, 10 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (1.05 g, 5 mmol), and the mixture was stirred under reflux for 4 hours. The reaction mixture was washed with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 10% methanol/chloroform to give 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethoxy}-ureido)-1-methylpyrazole (255 mg) as a solid.
^{1}H-NMR(DMSO-d_6) δ 1.38 (9H, s), 3.19-3.20 (2H, m), 3.51 (3H, s), 3.72 (2H, t, J=6Hz), 4.86 (2H, br), 6.95 (1H, br), 7.06 (1H, s), 8.02 (1H, brs), 9.15 (1H, brs)

Example 19

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethoxy)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethoxy}-ureido)-1-methylpyrazole in the same manner as in Example 17 as an amorphous solid.
^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 3.21 (1H, d, J=18Hz), 3.33 (2H, t, J=5Hz), 3.47 (1H, d, J=18Hz), 3.74 (3H, s), 4.17 (2H, t, J=5Hz), 4.99 (1H, d, J=15Hz), 5.17 (1H, d, J=15Hz), 5.26 (1H, d, J=5Hz), 5.86 (1H, d, J=5Hz), 7.93 (1H, s)

Preparation 36

To a suspension of 1,1'-carbonyldiimidazole (1.95 g, 12 mmol) in methylene chloride (20 ml) was added tert-butyl N-(2-aminoethyl)carbamate (1.92 g, 12 mmol) under ice-cooling, and the mixture was stirred at room temperature for 2 hours. To the reaction mixture were

added N-ethyldiisopropylamine (2.59 g, 20 mmol) and 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (2.22 g, 10 mmol), and the mixture was stirred at room temperature for 16 hours. To the reaction mixture were added trityl chloride (9.0 g, 32 mmol) and triethylamine (3.0 g, 30 mmol). The mixture was stirred at room temperature for 24 hours. The reaction mixture was washed with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 3% methanol/chloroform to give 7-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-2,3-dihydro-1-tritylimidazo[1,2-b]pyrazole (800 mg) as a solid.
^{1}H-NMR($CDCl_3$) δ 1.43 (9H, s), 3.19 (4H, br), 3.69 (1H, brs), 3.78-3.85 (4H, m), 4.51 (1H, br), 5.07 (1H, br), 7.20 (1H, s), 7.26-7.34 (9H, m), 7.46-7.47 (6H, m)

Example 20

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (820 mg, 1.0 mmol) in N,N-dimethylformamide (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-2,3-dihydro-1-tritylimidazo[1,2-b]pyrazole (700 mg, 1.2 mmol) and the whole mixture was stirred at room temperature for 6 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (120 ml), and the resulting precipitate was collected by filtration and

dried in vacuo. To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml). The resulting solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (120 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (830 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{7-[3-(2-aminoethyl)ureido]-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)}methyl-3-cephem-4-carboxylate (28.5 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.53 (3H, s), 1.54 (3H, s), 3.14 (2H, t, J=6Hz), 3.29 (1H, d, J=18Hz), 3.49 (2H, t, J=6Hz), 3.57 (1H, d, J=18Hz), 4.16 (2H, t, J=9Hz), 4.31-4.45(2H, m), 4.94 (1H, d, J=15Hz), 5.02 (1H, d, J=15Hz), 5.27 (1H, d, J=5Hz), 5.85 (1H, d, J=5Hz), 7.95 (1H, s)

Preparation 37

To a suspension of 1,1'-carbonyldiimidazole (2.0 g, 12.3 mmol) in dehydrated chloroform (30 ml) was added a solution of tert-butyl N-(2-hydroxyethyl)carbamate (1.92 g, 12 mmol) in dehydrated chloroform (10 ml) under ice-cooling, and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyldiisopropylamine (2.2 ml, 12.3 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (2.58 g, 12.3 mmol), and the mixture was stirred at room temperature for 17.5 hours. To the reaction mixture were added trityl chloride (3.42 g, 12.3 mmol) and triethylamine (1.25 g, 12.3 mmol). The mixture was

stirred at room temperature for 2 hours. The reaction mixture was washed with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 4-{[2-(tert-butoxycarbonylamino)ethoxycarbonyl]amino}-5-(tritylamino)-1-methylpyrazole (1.91 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.46 (9H, s), 2.89 (3H, s), 3.30-3.36 (2H, m), 4.03-4.07 (2H, m), 4.37 (1H, brs), 4.75 (1H, br), 5.42 (1H, br), 7.17-7.30 (16H, m)

Example 21

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethoxycarbonyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-{[2-(tert-butoxycarbonylamino)ethoxycarbonyl]amino}-5-(tritylamino)-1-methylpyrazole in the same manner as in Example 20 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.53 (3H, s), 1.54 (3H, s), 3.18 (1H, d, J=18Hz), 3.30-3.38 (2H, m), 3.43 (1H, d, J=18Hz), 3.71 (3H, s), 4.37-4.40 (2H, m), 4.97 (1H, d, J=15Hz), 5.18 (1H, d, J=15Hz), 5.24 (1H, d, J=5Hz), 5.83 (1H, d, J=5Hz), 7.95 (1H, s)

Preparation 38

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (1.42 g, 6.4 mmol) and N-ethyldiisopropylamine (2.73 g, 21 mmol) in methylene chloride (50 ml) was added N-[2-(tert-butoxycarbonylamino)acetoxy]succinimide (1.90 g, 7.0 mmol). The mixture was stirred at room temperature for 22 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution and

the organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 7-[2-(tert-butoxycarbonylamino)acetyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.07 g) as a solid.
^{1}H-NMR($CDCl_3$) δ 1.47 (9H, s), 3.89 (2H, d, J=5.5Hz), 3.97 (2H, dt, J=2.7Hz, 7.3Hz), 4.18 (2H, t, J=7.3Hz), 4.55 (1H, br), 5.22 (1H, br), 7.16 (1H, s), 7.95 (1H, br)

Example 22

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.2 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide (181 mg, 1.2 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-[2-(tert-butoxycarbonylamino)acetyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (421 mg, 1.5 mmol). The whole mixture was stirred at 30°C for 3 hours. To the resulting reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (150 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml). The resulting solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (150 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (830 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to

about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(2-aminoacetamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (20.8 mg) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (3H, s), 1.52 (3H, s), 3.26 (2H, d, J=18Hz), 3.54 (2H, d, J=18Hz), 3.97 (2H, s), 4.16 (2H, t, J=9Hz), 4.35 (1H, q, J=9Hz), 4.44 (1H, q, J=9Hz), 4.97 (2H, d, J=15Hz), 5.04 (2H, d, J=15Hz), 5.25 (1H, d, J=4Hz), 5.84 (1H, d, J=4Hz), 8.10 (1H, s)

Preparation 39

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (1.20 g, 5 mmol) and N-[2-(tert-butoxycarbonylamino)acetoxy]-succinimide (1.35 g, 5 mmol) in methylene chloride (20 ml) was added N-ethyldiisopropylamine (2.1 ml, 12 mmol) under ice-cooling, and the mixture was stirred at room temperature for 17 hours. The reaction mixture was washed with water (40 ml), saturated aqueous sodium hydrogen carbonate solution (40 ml) and brine (40 ml). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 10% methanol/chloroform to give 5-amino-4-[2-(tert-butoxycarbonylamino)acetyl]amino-1-(2-hydroxyethyl)pyrazole (1.20 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.46 (9H, s), 3.89-3.90 (4H, m), 4.00-4.04 (2H, m), 4.26 (2H, br), 5.51 (1H, br), 7.17 (1H, s), 8.06 (1H, br)

Example 23

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(2-aminoacetamido)-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-

cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[2-(tert-butoxycarbonylamino)acetyl]amino-1-(2-hydroxyethyl)pyrazole in the same manner as in Example 22 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (6H, s), 3.15 (2H, d, J=18Hz), 3.48 (2H, d, J=18Hz), 3.88 (1H, dt, J=16Hz), 4.02 (2H, s), 4.42 (1H, dt, J=16.5Hz), 5.07 (2H, d, J=15Hz), 5.13 (2H, d, J=15Hz), 5.27 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 8.09 (1H, s)

Preparation 40

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.96 g, 10 mmol) and N-ethyldiisopropylamine (2.59 g, 20 mmol) in methylene chloride (70 ml) was added N-[2-(tert-butoxycarbonylamino)acetoxy]succinimide (2.72 g, 10 mmol). The mixture was stirred at room temperature for 14 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 6% methanol/chloroform to give 3-[2-(tert-butoxycarbonylamino)acetyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (2.4 g) as a solid.

^{1}H-NMR($CDCl_3$) δ 1.46 (9H, s), 2.08-2.12 (2H, m), 3.29-3.32 (2H, m), 3.90 (2H, br), 4.07 (2H, t, J=6.0Hz), 5.00 (1H, br), 5.38 (1H, br), 7.12 (1H, s), 8.11 (1H, br)

Example 24

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-(2-aminoacetamido)-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-

3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-[2-(tert-butoxycarbonylamino)acetyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine in the same manner as in Example 22 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.52 (3H, s), 1.53 (3H, s), 2.05-2.25 (2H, m), 3.21 (2H, d, J=18Hz), 3.45 (2H, d, J=18Hz), 3.30-3.45 (2H, m), 4.00 (2H, s), 4.10-4.25 (2H, m), 4.92 (2H, d, J=15Hz), 5.17 (2H, d, J=15Hz), 5.24 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 7.97 (1H, s)

Preparation 41

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (4.44 g, 15 mmol) and N-ethyldiisopropylamine (3.88 g, 30 mmol) in methylene chloride (100 ml) was added N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (4.29 g, 15 mmol). The mixture was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 3-[3-(tert-butoxycarbonylamino)propionyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (3.67 g) as an oil.

^{1}H-NMR($CDCl_3$) δ 1.43 (9H, s), 2.08-2.13 (2H, m), 2.52 (2H, t, J=6.0Hz), 3.32 (2H, t, J=5.0Hz), 3.43-3.46 (2H, m), 4.07 (2H, t, J=6.0Hz), 5.12 (1H, br), 5.23 (1H, br), 7.13 (1H, s), 7.97 (1H, br)

Example 25

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-(3-aminopropionamido)-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-

[3-(tert-butoxycarbonylamino)propionyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine in the same manner as in Example 22 as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.51 (3H, s), 1.52 (3H, s), 2.05-2.25 (2H, m), 2.85 (2H, t, J=7Hz), 3.20 (2H, d, J=18Hz), 3.44 (2H, d, J=18Hz), 3.30-3.45 (2H, m), 3.31 (2H, t, J=7Hz), 4.05-4.20 (2H, m), 4.91 (2H, d, J=16Hz), 5.16 (2H, d, J=16Hz), 5.23 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 7.92 (1H, s)

Preparation 42

To a solution of 5-amino-1-methylpyrazole (100 g) in water (700 ml) were added concentrated hydrochloric acid (86 ml) and sodium nitrite (63.9 g) in water (200 ml) at a temperature below 10°C. The reaction mixture was stirred at 5°C for 30 minutes. The precipitated solid was collected by filtration and dried to give 5-amino-1-methyl-4-nitrosopyrazole (117 g).

^{1}H-NMR(DMSO-d_6) δ 3.52 and 3.59 (3H, s), 7.22 and 8.51 (1H, s), 8.17 and 8.51 (1H, brs)

Preparation 43

To a suspension of 5-amino-1-methyl-4-nitrosopyrazole (117 g) were added sulfuric acid (91 g) and 10% palladium on carbon (58 g). The mixture was hydrogenated under balloon pressure for 10 hours. The reaction mixture was filtered and the filtrate was concentrated in vacuo. To the concentrate was added isopropyl alcohol (2.3 L) and the mixture was stirred for 1 hour. The precipitated solid was collected by filtration and dried to give 4,5-diamino-1-methylpyrazole sulfuric acid salt (158 g).

^{1}H-NMR(D_2O) δ 3.74 (3H, s), 7.80 (1H, s)

Preparation 44

A solution of 4,5-diamino-1-methylpyrazole sulfuric acid salt (158 g) in water (1.1 L) was neutralized to pH 6.9 with 4N aqueous sodium hydroxide solution, and dioxane (474 ml) was added to this solution. To the resulting mixture was added dropwise phenyl chloroformate (124 g) maintaining pH of the

mixture at 6.9 with 4N aqueous sodium hydroxide solution at a temperature below 10°C. The reaction mixture was stirred for 1 hour. The precipitated solid was collected by filtration and dried to give 5-amino-1-methyl-4-phenoxycarbonylaminopyrazole (155 g).
^{1}H-NMR(DMSO-d_6) δ 3.52 (3H, s), 5.00 (2H, brs), 7.10-7.50 (6H, m), 8.93 (1H, brs)

Preparation 45

To a suspension of 5-amino-1-methyl-4-phenoxycarbonylaminopyrazole (153.8 g) in tetrahydrofuran (1 L) were added triethylamine (67 g) and triphenylmethyl chloride (185 g) at room temperature. The mixture was stirred for 6.5 hours. To the reaction mixture was added heptane (2.6 L) and the mixture was stirred for 1 hour. The precipitated solid was collected by filtration and washed with heptane-diisopropyl ether (1:1). The crude solid was suspended in water (3 L) and the suspension was stirred for 1 hour. The solid was collected by filtration and dried to give 1-methyl-4-phenoxycarbonylamino-5-triphenylmethylaminopyrazole (253.6 g).
^{1}H-NMR(DMSO-d_6) δ 2.74 (3H, s), 5.57 (1H, brs), 7.00-7.50 (21H, m), 8.12 (1H, brs)

Preparation 46

To a suspension of 1-methyl-4-phenoxycarbonylamino-5-triphenylmethylaminopyrazole (253.6 g) in N,N-dimethylformamide (1.5 L) were added triethylamine (59.5 g) and tert-butyl N-(2-aminoethyl)carbamate (94.2 g) in N,N-dimethylformamide (254 ml). The mixture was stirred for 5 hours and poured into water (10.6 L). The slurry was stirred for 1 hour. The precipitated solid was collected by filtration and dried to give a crude product. The crude product was suspended in N,N-dimethylformamide and the suspension was heated under reflux for 20 minutes. The suspension was cooled to ambient temperature over 4 hours. The solid was collected by filtration, washed with acetonitrile and dried to give 4-[N-(2-tert-

butoxycarbonylaminoethyl)carbamoylamino]-1-methyl-5-triphenylmethylaminopyrazole (261.2 g).

^{1}H-NMR(DMSO-d_6) δ 2.69 (3H, s), 2.90-3.05 (4H, m), 5.69 (1H, brs), 5.91-6.01 (1H, m), 6.74-6.81 (1H, m), 6.87 (1H, brs), 7.00 (1H, s), 7.10-7.30 (15H, m)

Preparation 47

To a solution of (Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetic acid (319 g, 642 mmol) in N,N-dimethylacetamide (1.5 L) were added potassium carbonate (113 g) and methanesulfonyl chloride (126 ml) under ice-cooling. The mixture was stirred at 10°C for 2 hours. The reaction mixture was added to a mixture of ethyl acetate and water. The organic layer was washed with water and brine to give an activated acid solution. On the other hand, a suspension of 4-methoxybenzyl 7β-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride (300 g, 740 mmol) in a mixture of water (1 L) and ethyl acetate (1 L) was adjusted to pH 6 with triethylamine under ice-cooling. To the resulting mixture was dropwise added the above obtained activated acid solution at 10°C under stirring. Stirring was continued at 5-10°C for 1.5 hours keeping pH of the reaction mixture at 6 with triethylamine. The organic layer was separated, washed with water and brine, and evaporated in vacuo. The concentrate was poured into diisopropyl ether (15 L), and the resulting precipitate was collected by filtration and dried to give 4-methoxybenzyl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (495.7 g, yield 98.3%).

^{1}H-NMR(DMSO-d_6) δ 1.39 (9H, s), 1.44 (6H, s), 3.45-3.70 (2H, m), 3.76 (3H, s), 4.46 and 4.54 (1H, ABq, J=16Hz), 5.10-5.28 (2H+1H, m), 5.90 (1H, dd, J=4.9Hz, 8.5Hz), 6.94 (2H, d, J=8.7Hz), 7.36 (2H, d, J=8.7Hz), 8.18 (2H, brs), 9.52 (1H, d, J=8.5Hz)

Example 26

To a solution of 4-methoxybenzyl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (150 g) in N,N-dimethylformamide (400 ml) was added 1,3-bis(trimethylsilyl)urea (225 g) and the mixture was stirred for 30 minutes. Potassium iodide (51.2 g) was added to this solution and the mixture was stirred for 30 minutes.

4-[N-(2-tert-Butoxycarbonylaminoethyl)-carbamoylamino]-1-methyl-5-triphenylmethylaminopyrazole (147 g) was dissolved in N,N-dimethylformamide (650 ml) at 78°C and the solution was cooled to 45°C. The solution was added to the solution of 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate obtained above. The reaction mixture was stirred at 35°C for 18.5 hours and poured into a mixture of ethyl acetate (2 L), water (1.8 L) and 20% aqueous sodium chloride solution (150 ml). The organic layer was washed with a mixture of 10% aqueous sodium thiosulfate solution (375 ml) and 20% aqueous sodium chloride solution (375 ml). The organic layer was washed successively with 10% aqueous sodium trifluoroacetate solution three times (750 ml x 3) and 20% aqueous sodium chloride solution (750 ml). The organic layer was concentrated in vacuo and precipitated 4-[N-(2-tert-butoxycarbonylaminoethyl)carbamoylamino]-1-methyl-5-triphenylmethylaminopyrazole was filtered off. The filtrate was further concentrated in vacuo to a volume of approximately 600 ml. This solution was added to diisopropyl ether and the suspension was stirred for 1 hour. The resulting solid was collected by filtration and dried. The solid was dissolved in dichloromethane (669 ml). To the solution were added anisole (223 ml) and trifluoroacetic acid (669 ml). The reaction mixture was stirred for 4 hours and poured into diisopropyl ether. The resulting solid was collected by filtration and dried. This solid was suspended in water and pH of

the suspension was adjusted to 7 with aqueous ammonia solution at a temperature below 10°C. The resulting precipitate was filtered off. The filtrate was acidified to pH 1 with concentrated hydrochloric acid at a temperature below 10°C and the resulting precipitate was filtered off. The filtrate was chromatographed on Diaion® HP-20 (11 L) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.5 L in vacuo and 2M sulfuric acid (51 ml) was added. The mixture was lyophilized to give crude product (72.2 g).

The crude product (3 g) was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (400 ml) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 73 ml in vacuo and 2M sulfuric acid (1.5 ml) was added. The mixture was further evaporated to a volume of approximately 12.5 ml and water (6 ml) was added. After addition of seed crystals (10 mg), which resulted in the precipitation of a white solid, the mixture was stirred at room temperature for 1 hour. The mixture was further stirred at 5°C for 13 hours. 2-Propanol (20 ml) was added at 5°C over 20 minutes and the slurry was stirred at room temperature for 4 hours. 2-Propanol (20 ml) was added over 30 minutes and the slurry was stirred at room temperature for 3 hours. The precipitated crystals were collected by filtration and dried to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[N-(2-aminoethyl)carbamoylamino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate sulfate (1.51 g) as crystals.

^{1}H-NMR(D_2O) δ 1.61 (6H, s), 3.10-3.55 (6H, m), 3.71 (3H, s), 5.02 and 5.23 (2H, ABq, J=16.7Hz), 5.25 (1H, d, J=4.9Hz), 5.87 (1H, d, J=4.9Hz), 7.91 (1H, s)

Preparation 48

A suspension of 4,5-diamino-1-methylpyrazole sulfuric acid salt (20 g, 95.1 mmol) in triethylamine (29.2 ml, 209 mmol) was stirred at 0°C for 10 minutes. A mixture of acetic anhydride (9.87 ml, 105 mmol) and formic acid (7.96 ml, 209 mmol) was stirred at 40°C for 30 minutes, cooled to 0°C, and added dropwise to the above solution at 0°C. The whole mixture was stirred at 0°C for 2 hours. To the mixture was added brine, and the whole mixture was extracted with tetrahydrofuran. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure to give crude N-(5-amino-1-methyl-1H-pyrazol-4-yl)formamide, which was used in the next step without further purification.

Preparation 49

The crude product of N-(5-amino-1-methyl-1H-pyrazol-4-yl)formamide (13.33 g, 95.1 mmol) was dissolved in N,N-dimethylformamide (130 ml). To the solution were added trityl chloride (29.2 g, 105 mmol), triethylamine (66.3 ml, 476 mmol) and 4-dimethylaminopyridine (465 mg, 3.8 mmol), and the mixture was stirred at 60°C for 5 hours. To the reaction mixture was added water, and the whole mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to give a white solid. The solid was triturated with ethyl acetate/diisopropyl ether (1:1) to give N-[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]formamide (19.18 g). The NMR spectrum of this compound indicates the existence of its rotamer.

^{1}H-NMR(DMSO-d_6) δ 2.76 and 2.92 (3H, s), 5.56 and 5.84 (1H, s), 7.0-7.4 (16H, m), 7.66 (1H, d, J=1.7Hz), 8.3-8.4 (1H, m)

ESI-MS: m/z=405.2$(M+Na)^+$

Preparation 50

To a solution of N-[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]formamide (3.825 g, 10 mmol) in N,N-dimethylformamide (66 ml) was added sodium hydride (264

mg, 60% oil suspension, 11 mmol) under a nitrogen atmosphere at 0°C under stirring. The mixture was stirred at 0°C for 15 minutes. To the mixture were added tert-butyl N-(3-bromopropyl)carbamate (2.62 g, 11 mmol) in N,N-dimethylformamide (10 ml) and sodium iodide (1.65 g, 11 mmol). The mixture was warmed to room temperature and stirred for 2 hours. 10% Aqueous potassium hydrogen sulfate solution (5 ml) was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel eluting with methylene chloride/ethyl acetate (4:1) to give tert-butyl 3-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}propylcarbamate (2.714 g, yield 50.3%). The NMR spectrum of this compound indicates the existence of its rotamer.

^{1}H-NMR(DMSO-d_6) δ 1.37 and 1.39 (9H, s), 2.6-2.9 (6H, m), 2.89 (3H, s), 5.34 and 6.01 (1H, s), 6.6-6.9 (1H, m), 7.0-7.4 (15H, m), 7.5-7.6 (1H, m), 7.95 (1H, s)

ESI-MS: m/z=562.3 $(M+Na)^+$

Example 27

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(3-aminopropyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and tert-butyl 3-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}propylcarbamate in the same manner as in Example 1. The NMR spectrum of this compound indicates the existence of its rotamer.

^{1}H-NMR(D_2O) δ 1.53 (6H, s), 1.7-2.1 (2H, m), 2.9-3.9 (9H, m), 4.97 and 5.20 (2H, ABq, J=15.2Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.0-8.3 (2H, m)

Example 28

To a suspension of 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)-acetamido]-3-{3-amino-4-[(3-aminopropyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (140 mg, 0.21 mmol) in methanol (2.6 ml) was added concentrated hydrochloric acid (0.176 ml, 2.1 mmol) at room temperature, and the mixture was stirred for 6.5 hours. To the reaction mixture was added sodium hydrogen carbonate (177 mg, 2.1 mmol), and the mixture was purified by preparative HPLC (ODS column, acetonitrile/phosphate buffer (pH 7)=5:95). The eluate containing a desired product was evaporated to remove acetonitrile, acidified with diluted hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated under reduced pressure and freeze-dried to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(3-aminopropyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (39 mg).

^{1}H-NMR(D_2O) δ 1.52-1.54 (6H, m), 1.95 (2H, tt, J=7.3Hz, 7.3Hz), 3.0-3.2 (4H, m), 3.16 and 3.38 (2H, ABq, J=17.7Hz), 3.68 (3H, s), 4.89 and 5.11 (2H, ABq, J=15.6Hz), 5.22 (1H, d, J=4.8Hz), 5.83 (1H, d, J=4.8Hz), 7.59 (1H, s)

ESI-MS: m/z=636.3$(M-H)^-$

Preparation 51

tert-Butyl 2-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}ethylcarbamate

The title compound was obtained from N-[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]formamide and tert-butyl N-(2-bromoethyl)carbamate in the same manner as in Preparation 50.

IR(KBr) 1709, 1670, 1170, 704 cm^{-1}

^{1}H-NMR(DMSO-d_6) δ 1.35 and 1.36 (9H, s), 2.65 and 2.75 (3H, s), 2.73-2.90 (4H, m), 5.45 and 6.02 (1H, s), 6.78 and 6.88 (1H, t-like), 7.05-7.30 (15H, m), 7.31 and 7.57

(1H, s)

ESI-MS: m/z=426.3($M+H^+$), 548.3($M+Na^+$)

Example 29

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate and tert-butyl 2-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}ethylcarbamate in the same manner as in Example 1.

IR(KBr) 1770, 1675, 1653, 1597 cm^{-1}

^1H-NMR(DMSO-d_6) δ 1.53 (6H, s), 3.12-3.78 (4H, m), 3.77 and 3.78 (3H, s), 3.86-3.96 (2H, m), 5.00 and 5.19 (2H, ABq, J=15.2Hz), 5.28 (1H, d, J=4.8Hz), 5.86 (1H, d, J=4.8Hz), 8.15 and 8.18 (1H, s), 8.19 and 8.33 (1H, s)

ESI-MS: m/z=652.2($M+H^+$)

Example 30

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate in the same manner as in Example 28.

IR(KBr) 1770, 1651, 1593 cm^{-1}

^1H-NMR(DMSO-d_6) δ 1.53 (3H, s), 1.59 (3H, s), 3.13-3.26 (4H, m), 3.26 and 3.39 (2H, ABq, J=17.8Hz), 3.68 (3H, s), 4.87 and 5.11 (2H, ABq, J=15.7Hz), 5.25 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 7.63 (1H, s)

ESI-MS: m/z=622.2($M-H^-$)

Preparation 52

To a suspension of 1-methyl-1H-pyrazole-4,5-

diamine sulfate (86 g) in tetrahydrofuran (1.3 L) was added triethylamine (117 ml) and then (2S)-4-[(tert-butoxycarbonyl)amino]-2-hydroxybutanoic acid (82.5 g) was added to the mixture. To the mixture were added 1-hydroxybenzotriazole (58.3 g) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (82.7 g) under ice-cooling. The reaction mixture was stirred at room temperature for 8 hours. To the reaction mixture were added ethyl acetate (1.3 L), saturated aqueous sodium hydrogen carbonate solution and sodium chloride and the mixture was stirred for 30 minutes. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (1.0 L) six times. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with ethyl acetate/tetrahydrofuran (1/1) to give tert-butyl {(3S)-4-[(5-amino-1-methyl-1H-pyrazol-4-yl)amino]-3-hydroxy-4-oxobutyl}carbamate (69.5g).

^{1}H-NMR($CDCl_3$) δ 1.43 (9H, s), 1.6-1.9 (1H, m), 1.9-2.2 (1H, m), 3.1-3.3 (1H, m), 3.3-3.5 (1H, m), 3.65 (3H, s), 4.20 (1H, dd, J=3.6, 6.6Hz), 4.7-5.3 (4H, m), 7.24 (1H, s), 8.58 (1H, s)

$[\alpha]^{20}_D$(c=1.05, $CHCl_3$)=-27.06°

Preparation 53

To a solution of tert-butyl {(3S)-4-[(5-amino-1-methyl-1H-pyrazol-4-yl)amino]-3-hydroxy-4-oxobutyl}carbamate (68.51 g) in N,N-dimethylformamide (350 ml) was added chlorotriphenylmethane (67 g). To the mixture was dropwise added triethylamine (67 ml). The mixture was stirred at room temperature for 12 hours. The reaction mixture was dissolved in dichloromethane (2 L). The solution was washed successively with water and brine. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with acetonitrile and dried in vacuo to give tert-butyl ((3S)-3-hydroxy-4-{[1-methyl-5-

(tritylamino)-1H-pyrazol-4-yl]amino}-4-oxobutyl)carbamate (64 g).

^{1}H-NMR($CDCl_3$) δ 1.46 (9H, s), 1.3-1.6 (1H, m), 1.8-2.1 (1H, m), 2.95 (3H, s), 2.9-3.2 (1H, m), 3.3-3.6 (1H, m), 3.95 (1H, m), 4.53 (1H, d, J=4.5Hz), 4.74 (1H, s), 4.92 (1H, brs), 7.1-7.3 (15H, m), 7.39 (1H, s), 7.73 (1H, s)

ESI-MS: m/z=638.2($M+H+Na^+$)

$[\alpha]^{20}_D$(c=1.025, $CHCl_3$)=-36.5°

Example 31

To a solution of 4-methoxybenzyl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (130 g) in N,N-dimethylformamide (400 ml) was added 1,3-bis(trimethylsilyl)urea (195 g) and the mixture was stirred at room temperature for 30 minutes. To the solution was added potassium iodide (44.4 g) and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added tert-butyl ((3S)-3-hydroxy-4-{[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}-4-oxobutyl)carbamate (106 g) and the whole mixture was stirred at 35°C for 22 hours. To the reaction mixture was added ethyl acetate (1.7 L) and the mixture was washed successively with water (1.6 L), 10% aqueous sodium trifluoroacetate solution (650 ml x 3) and brine (650 ml), dried over magnesium sulfate and filtered. The filtrate was concentrated to about 1 L in vacuo. The concentrate was poured into diisopropyl ether (3 L) and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the solid in methylene chloride (660 ml) were added anisole (220 ml) and trifluoroacetic acid (660 ml).

The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether (7 L). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (156.2 g). The crude product was dissolved in water (3.5 L). The solution was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on

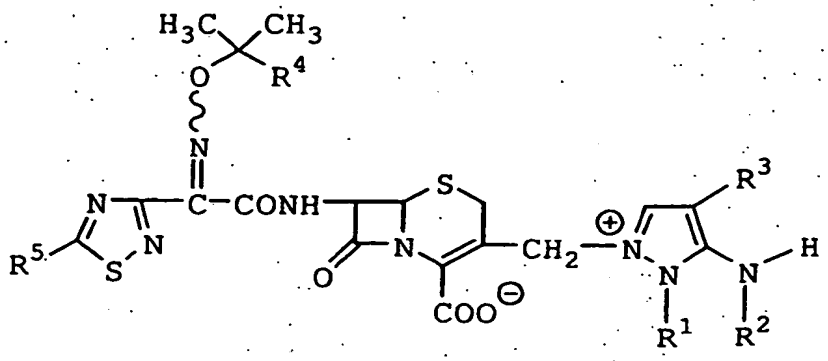
Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 1.5 L in vacuo and 2M aqueous sulfuric acid solution (33.18 ml) was added. The mixture was lyophilized. The lyophilized product (40 g) was dissolved in phosphate buffer (pH 7) and purified by preparative HPLC utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 10% aqueous 2-propanol. The eluate was concentrated to about 1 L in vacuo and 2M aqueous sulfuric acid solution was added (13.59 ml). The resulting solution was lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[((2S)-4-amino-2-hydroxybutanoyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate sulfate (20.82 g, yield 14%) as an amorphous solid.

^{1}H-NMR(D_2O) δ 1.61 (6H, s), 1.9-2.4 (2H, m), 3.20 (1H, d, J=17.6Hz), 3.0-3.3 (2H, m), 3.45 (1H, d, J=17.6Hz), 3.74 (3H, s), 4.47 (1H, dd, J=4, 6.3Hz), 5.06 (1H, d, J=15.7Hz), 5.25 (1H, d, J=4.8Hz), 5.28 (1H, d, J=15.7Hz), 5.87 (1H, d, J=4.8Hz), 8.07 (1H, s)

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound of the formula [I]:



[I]

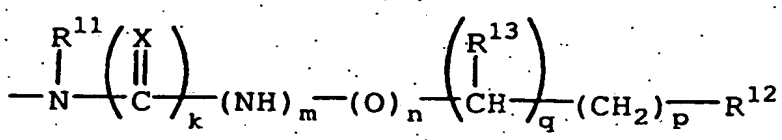
wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen or amino protecting group, or

R^1 and R^2 are bonded together and form lower alkylene;

R^3 is



wherein X is O or NH,

R^{11} is hydrogen or amino protecting group,

R^{12} is amino or protected amino,

R^{13} is hydrogen or hydroxy,

k, m, n and q are independently 0 or 1, and

p is 0, 1 or 2;

R^4 is carboxy or protected carboxy; and

R^5 is amino or protected amino,

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen, aryl(lower)alkyl or acyl, or

R^1 and R^2 are bonded together and form lower alkylene;

R^4 is carboxy or esterified carboxy;

R^5 is amino or acylamino;

R^{11} is hydrogen or acyl; and

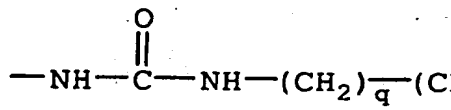
R^{12} is amino or acylamino,

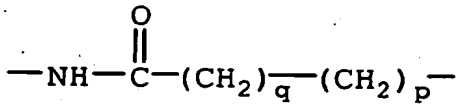
or a pharmaceutically acceptable salt thereof.

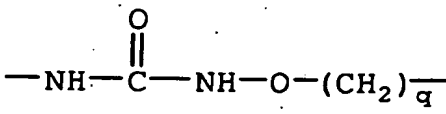
3. The compound of claim 2 wherein
R^1 is lower alkyl or hydroxy(lower)alkyl, and
R^2 is hydrogen, aryl(lower)alkyl, lower alkanoyl or lower alkoxycarbonyl, or
R^1 and R^2 are bonded together and form lower alkylene;
R^4 is carboxy or lower alkoxycarbonyl;
R^5 is amino, lower alkanoylamino or lower alkoxycarbonylamino;
R^{11} is hydrogen, lower alkanoyl or lower alkoxycarbonyl; and
R^{12} is amino, lower alkanoylamino or lower alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

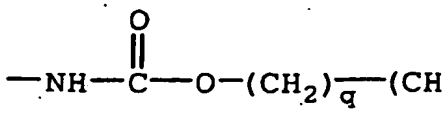
4. The compound of claim 3 wherein
R^1 is lower alkyl or hydroxy(lower)alkyl, and
R^2 is hydrogen, or
R^1 and R^2 are bonded together and form lower alkylene;
R^4 is carboxy;
R^5 is amino;
R^{11} is hydrogen or lower alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 wherein
R^3 is selected from the group consisting of

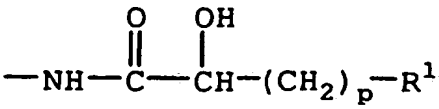




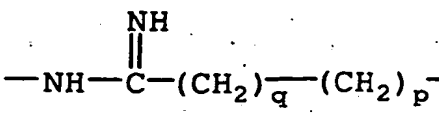




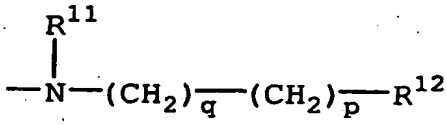
,



,



and

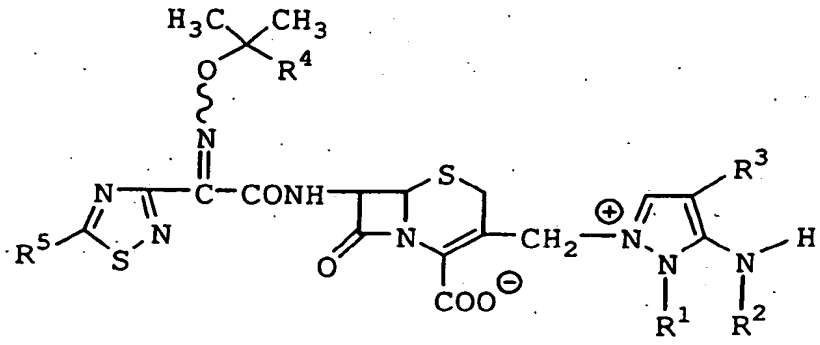


wherein R^{11}, R^{12}, p and q are each as defined in claim 1, or a pharmaceutically acceptable salt thereof.

6. The compound of claim 5 wherein
R^{11} is hydrogen, lower alkanoyl or lower alkoxycarbonyl; and
R^{12} is amino, lower alkanoylamino or lower alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

7. The compound of claim 6 wherein
R^{11} is hydrogen or lower alkanoyl; and
R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

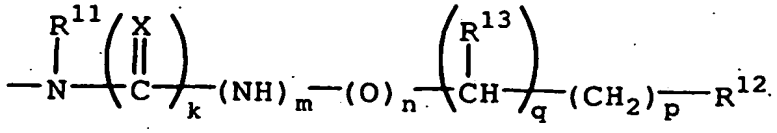
8. A process for preparing a compound of the formula [I]:



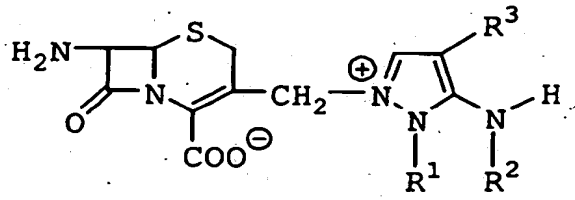
[I]

wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and
R^2 is hydrogen or amino protecting group, or
R^1 and R^2 are bonded together and form lower alkylene;
R^3 is

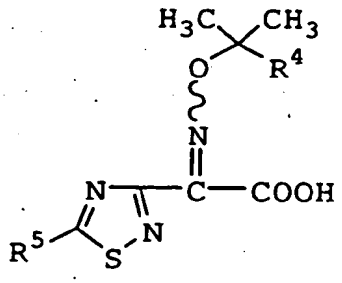


wherein X is O or NH,
R^{11} is hydrogen or amino protecting group,
R^{12} is amino or protected amino,
R^{13} is hydrogen or hydroxy,
k, m, n and q are independently 0 or 1, and
p is 0, 1 or 2;
R^4 is carboxy or protected carboxy; and
R^5 is amino or protected amino,
or a salt thereof, which comprises
(1) reacting a compound of the formula [II]:



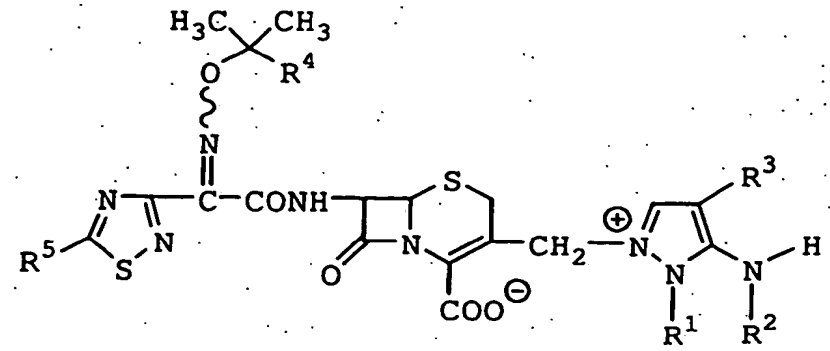
[II]

wherein R^1, R^2 and R^3 are each as defined above, or its reactive derivative at the amino group, or a salt thereof with a compound of the formula [III]:



[III]

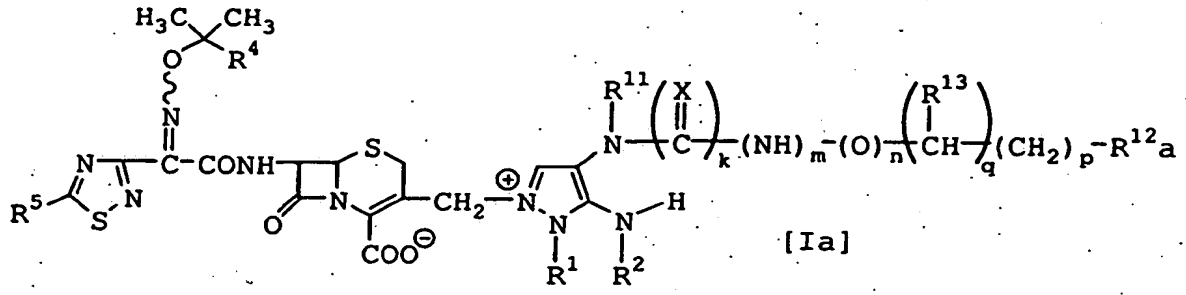
wherein R^4 and R^5 are each as defined above, or its reactive derivative at the carboxy group, or a salt thereof to give a compound of the formula [I]:



[I]

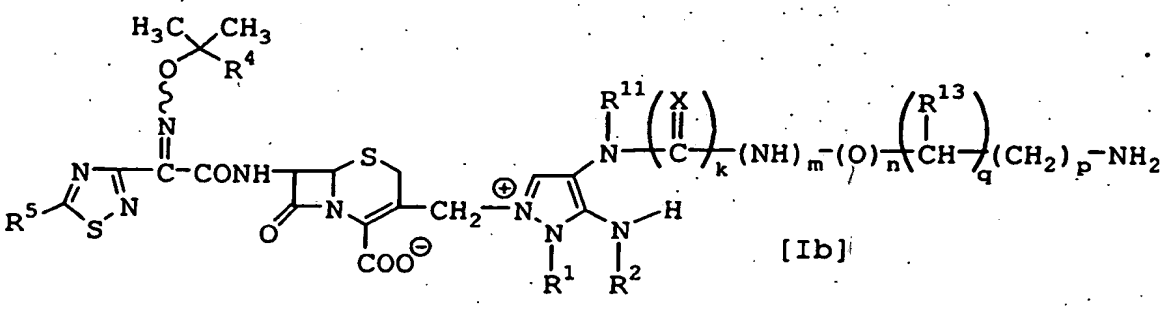
wherein R^1, R^2, R^3, R^4 and R^5 are each as defined above, or a salt thereof, or

(2) subjecting a compound of the formula [Ia]:



[Ia]

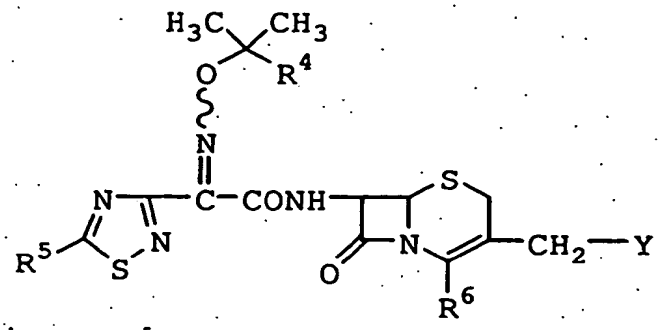
wherein R^1, R^2, R^4, R^5, R^{11}, R^{13}, X, k, m, n, p and q are each as defined above, and $R^{12}a$ is protected amino, or a salt thereof to elimination reaction of the amino protecting group to give a compound of the formula [Ib]:



[Ib]

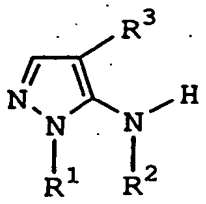
wherein R^1, R^2, R^4, R^5, R^{11}, R^{13}, X, k, m, n, p and q are each as defined above, or a salt thereof, or

(3) reacting a compound of the formula [VI]:



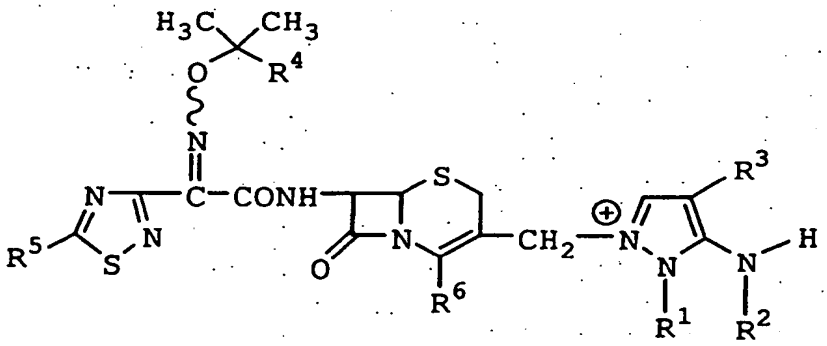
[VI]

wherein R^4 and R^5 are each as defined above, R^6 is protected carboxy, and Y is a leaving group, or a salt thereof with a compound of the formula [VII]:



[VII]

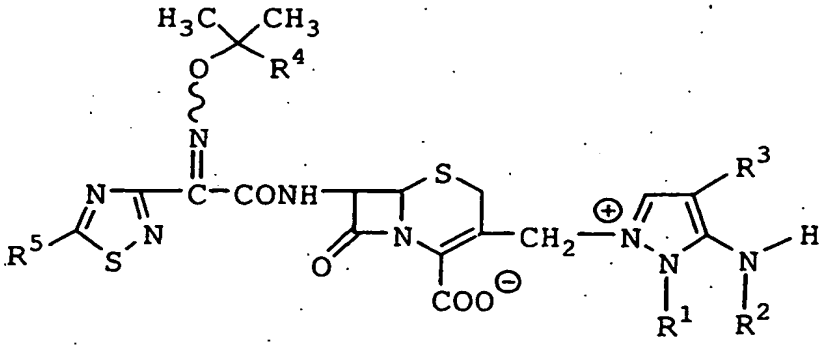
wherein R^1, R^2 and R^3 are each as defined above, or a salt thereof to give a compound of the formula [VIII]:



[VIII]

wherein R^1, R^2, R^3, R^4, R^5 and R^6 are each as defined above, or a salt thereof, and

subjecting the compound of the formula [VIII] or a salt thereof to elimination reaction of the carboxy protecting group, to give a compound of the formula [I]:



[I]

wherein R^1, R^2, R^3, R^4 and R^5 are each as defined above, or a salt thereof.

9. A pharmaceutical composition comprising a compound of claim 1 or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier.

10. A compound of claim 1 or a pharmaceutically acceptable salt thereof for use as a medicament.

11. A compound of claim 1 or a pharmaceutically acceptable salt thereof for use as an antimicrobial agent.

12. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof for manufacture of a medicament for treating infectious diseases.

13. A method for the treatment of infectious diseases which comprising administering a compound of claim 1 or a pharmaceutically acceptable salt thereof to human or animals.

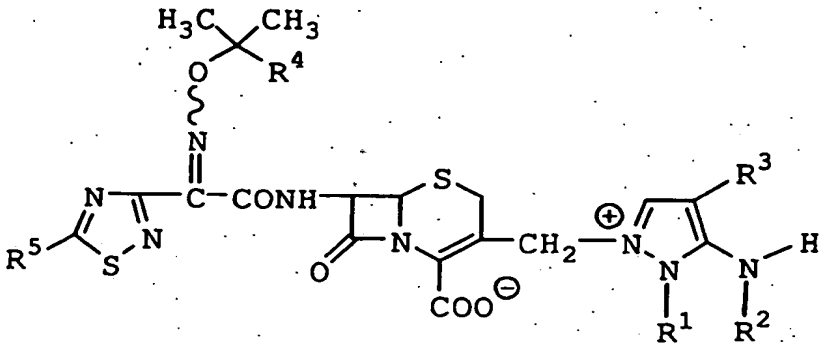
DATED this 4th day of September, 2003

Fujisawa Pharmaceutical Co., Ltd.
and
Wakunaga Pharmaceutical Co., Ltd.

By DAVIES COLLISON CAVE
Patent Attorneys for the Applicants

ABSTRACT

The present invention relates to a compound of the formula [I]:



[I]

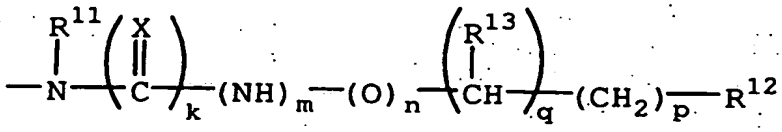
wherein

R^1 is lower alkyl or hydroxy(lower)alkyl, and

R^2 is hydrogen or amino protecting group, or

R^1 and R^2 are bonded together and form lower alkylene;

R^3 is



wherein X is O or NH,

R^{11} is hydrogen or amino protecting group,

R^{12} is amino or protected amino,

R^{13} is hydrogen or hydroxy,

k, m, n and q are independently 0 or 1, and

p is 0, 1 or 2;

R^4 is carboxy or protected carboxy; and

R^5 is amino or protected amino,

or a pharmaceutically acceptable salt thereof, a process for preparing a compound of the formula [I], and a pharmaceutical composition comprising a compound of the formula [I] in admixture with a pharmaceutically acceptable carrier.